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PENETRATION OF SILVER NITRATE INTO DENTIN II[1]

H. A. ZANDER AND H. W. SMITH

Tufts College Dental School, Boston, Mass.

In a previous publication (1) experiments were described which showed that silver nitrate solution penetrates sound dentin. The experiments also indicated that the product of the silver nitrate precipitation may possibly be carried into the pulp by a tissue fluid (dental lymph).

The purpose of this investigation was to determine whether the product of the silver nitrate[2] precipitation is carried into the pulp by a tissue fluid (dental lymph) or whether the penetration of silver nitrate simply follows physical laws governing the progress of fluid through capillary channels. The answer to this question could be found by comparing the penetration of silver nitrate into sound dentin, into degenerated dentin of vital teeth and into dentin of teeth without vital pulps. If there is a difference in the degree of penetration, it is likely that the product of silver nitrate precipitation is carried by the interstitial fluid. If, however, the degree of penetration is the same, silver nitrate penetration is not necessarily associated with the flow of a tissue fluid.

Experiments were carried out on 6 dogs and 5 humans. The dogs selected were about 1 year old, in order to reduce to a minimum any disturbances that may have taken place in the teeth due to increased age. Under sodium pentothal (Nembutal) anesthesia, cavities were prepared in the gingival third of the labial surfaces of the upper and lower anterior teeth under a constant stream of cold water. Entrance into the dentin through the enamel was obtained by the use of a rapidly revolving No. 227 knife-edge diamond stone. The remainder of the cavity preparation was accomplished with a No. 33½ inverted cone bur. All cavities were cut to approximately the same depth. Following preparation, the cavities were dried, treated with silver nitrate in some cases and filled with zinc oxyphosphte cement.

The purpose of these procedures was to produce degenerative processes in the dentinal tubules or fibers. It is known that any injury to the peripheral end of the tubules such as caries, abrasion, or cavity preparation results in dentin changes which produces what has been termed "sclerosed dentin" (2, 3), "metamorphosed dentin" (4, 5, 9) and "dead tracts" (6). These changes are described as degenerative processes. Associated with these processes is an interference with the metabolic phenomena in dentin (6, 8, 9).

[1] Received for publication February 26, 1945.

[2] The term silver nitrate has been used for Howe's Ammoniacal silver nitrate solution in this paper.

EXPERIMENTAL

I. Dogs as experimental subjects

Dog A: Cavities were prepared and filled on the first experimental dogs in the following teeth: U. R. 1, 2, 3, 4; U. L. 1, 2, 3, 4; L. R. 3, 4; L. L. 3. 4. Two months later these teeth were removed and fixed.

Dog B: Cavities were prepared in the following teeth: U. R. 1, 2, 3, 4, 6, 7; U. L. 1, 2, 3, 4, 6, 7; L. R. 1, 2, 3, 4; and L. L. 1, 2, 3, 4. These cavities were filled immediately after preparation with zinc oxyphosphate cement. One week later the teeth were removed and fixed.

Both dogs showed a narrowing of the predentin zone and irregular dentin formation adjacent to the injured tubules. These two dogs served as controls for the silver nitrate experiments and demonstrated that the disturbances in dentin (dead tracts) could be produced merely by these operative procedures. In order to demonstrate how the penetration of silver nitrate into such areas of degenerated dentin compares with its penetration into sound dentin, the following procedures were performed on dogs C, D, E, and F.

Dogs C and D: First day—Cavities were prepared and cement fillings placed in the following teeth: U. R. 3; U. L. 2, 3; L. R. 2; L. L. 1, 4. Thirtieth day—Cavities were prepared and cement fillings placed in the following teeth: U. L. 1, 4; L. L. 2, 3. Sixtieth day—Cavities were prepared and silver nitrate solution[2] applied and cement fillings placed in the following teeth: U. R. 1, 2, 4; L. R. 1, 3, 4. Cement fillings were removed and silver nitrate solution applied and cement fillings replaced on the following teeth: U. R. 3; U. L. 1, 2, 3, 4; L. R. 2; L. L. 1, 2, 3, 4. Sixty-seventh day—All teeth were removed and fixed immediately.

Dogs E and F: First day—Cavities were prepared and cement fillings placed in the following teeth: U. R. 4; U. L. 2, 3; L. R. 1; L. L. 1, 4. Sixth day—Cavities were prepared and cement fillings placed in the following teeth: U. L. 1, 4; L. L. 2, 3. Seventh day—Cavities were prepared and silver nitrate solution applied and cement fillings placed in the following teeth: U. R. 1, 2, 3; L. R. 2, 3, 4. Cement fillings removed and silver nitrate applied and cement fillings replaced in the following teeth: U. R. 4; U. L. 1, 2, 3, 4; L. R. 1; L. L. 1, 2, 3, 4.

These procedures enabled us to observe teeth to which silver nitrate had been applied after the degenerative changes had occurred in dentin, and teeth in which degenerative changes have not as yet occurred. The reason for the various intervals was to establish various degrees of dentin degeneration before silver nitrate application.

II. Human beings as experimental subjects

The same experimental procedures were carried out on 26 human teeth. Cavities were also prepared in pulpless human teeth and silver nitrate solution applied.

After the removal of the teeth, ground sections were prepared from some of the teeth while most of them were decalcified and sectioned by a method previously

described (1). The use of silver salts allows a comparison of ground and decalcified sections (10), thus showing soft tissue changes as well as hard tissue changes.

RESULTS

A. Control dogs

Among the 34 teeth in which cavities had been prepared and filled with zinc oxyphosphate cement there were 11 teeth which showed no changes at all in the dentin and pulp or only slight injury to the odontoblasts. The great majority of the teeth showed some degeneration of the odontoblastic layer manifested by narrowing of the predentin zone, and/or irregular dentin formation. These changes are shown in *fig. 1*, a photomicrograph of a decalcified section of a dog's tooth. The cavity (A) was cut into the dentin and filled for 7 days with zinc oxyphosphate cement before the animal was sacrificed. The reaction at the pulpal end of the tubules exposed by the cavity preparation can be seen at (D). A high magnification of this area can be seen in *fig. 2*. In this region the layer of predentin is narrowed; a phenomenon which is not due to an arrest of the normal predentin formation, but probably occurs because of a precipitation of calcium salts in that part of the predentin which is in contact with the injured tubules. The odontoblasts adjacent to this area appear slightly injured and reduced in height.

B. Silver Nitrate Dogs

Of the 40 teeth in which cavities had been prepared to cause degenerative changes before silver nitrate application, 29 showed injury to the odontoblasts, narrowing of the predentin zone and/or irregular dentin formation. The silver nitrate solution penetrated through the dentin (*fig. 3*), the "calcific barrier" (D) and the irregular dentin (E) into the pulp (C). In this case a cavity (A) had been exposed to silver nitrate after having been filled with zinc oxyphosphate cement for 2 months. An area of irregular dentin (E) which corresponds to the tubules injured in the cavity preparation, can be seen. Between the primary and irregular dentin a deeply stained line (D) appears. This line has been described by Fish and also by Manley (7) as a "calcific barrier" which they claim acts as a seal to the pulpal end of the injured tubules. Gurley and Van Huysen (11) referred to it as a "line of injury." In a high magnification of this "barrier" (*fig. 4*) the silver nitrate is seen to precipitate in the dentinal tubules on both sides of the line thus demonstrating that it is permeable. A high magnification (*fig. 5*) of the odontoblastic zone (F) from *fig. 3*, shows that the silver nitrate precipitate has penetrated through the "barrier," irregular dentin, and predentin to reach the pulp.

No correlation could be found between the changes in the pulp and dentin and the penetration of silver nitrate solution. If there was any difference at all between these and the control teeth,—that is those teeth which were silver nitrated immediately before any reactions on the part of the pulp or dentin had taken

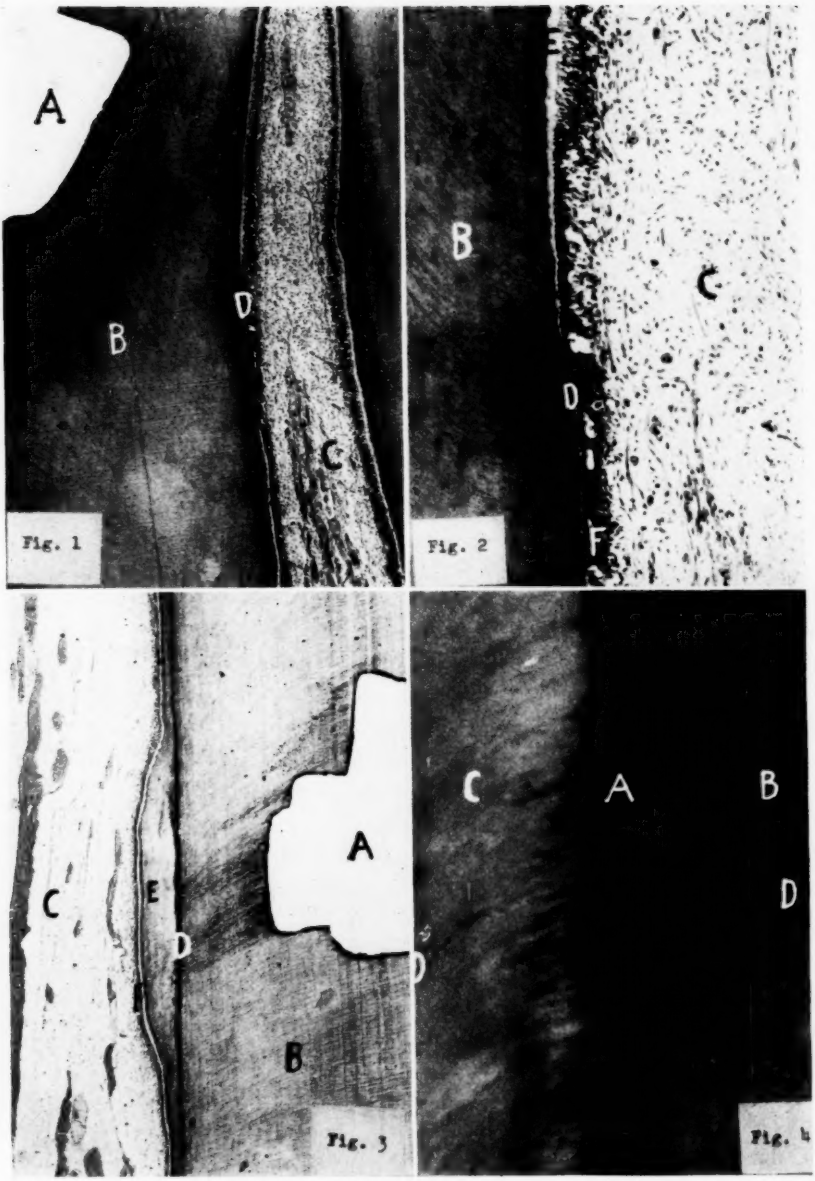

Fig. 1. Photomicrograph of dog's tooth in which cavity A was prepared and filled with zinc oxyphosphate cement for 7 days before animal was sacrificed. B—dentin, C—pulp, D—area of predentin corresponding to pulpal end of dentinal tubules affected by cavity preparation.

Fig. 2. High magnification of area D from *fig. 1.* B—dentin, C—pulp, D—zone of predentin corresponding to the injured tubules, E—predentin corresponding to uninjured dentinal tubules, F—odontoblastic layer.

Fig. 3. Photomicrograph of dog's tooth in which cavity A was prepared and filled with zinc oxyphosphate cement for 2 months. Filling was then removed, silver nitrate applied and filled again. Dog was sacrificed 1 week later. B—primary dentin, C—pulp, D—"calcific barrier," E—irregular dentin, F—odontoblastic layer.

Fig. 4. High magnification of area D in *fig. 3* showing both sides of "calcific barrier"at A. B—cavity side of "barrier," C—pulpal side of "barrier," D—silver precipitate in the tubules.

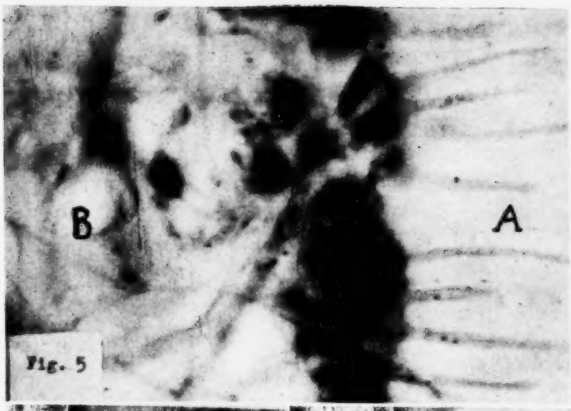

Fig. 5

Fig. 6(A)

Fig. 6(B)

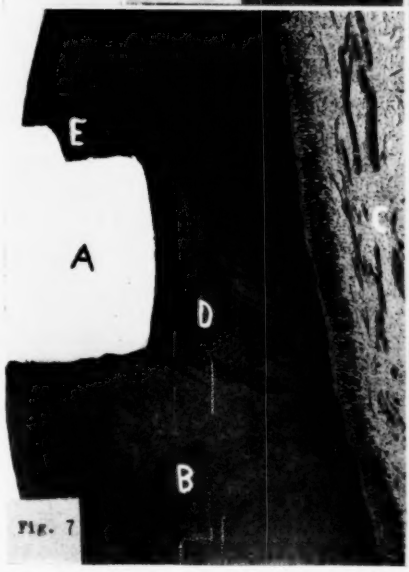

Fig. 7

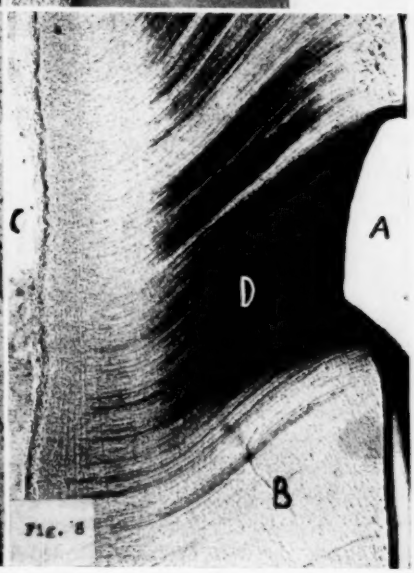

Fig. 8

Fig. 5. High magnification of odontoblastic layer, F, of *fig. 3*. A—dentin, B—pulp. Particles of product of silver nitrate precipitation can be seen in odontoblasts and pulp.

Fig. 6a. Photomicrograph of a dog's tooth in which cavity A had been prepared, silver nitrate solution applied and then filled with zinc oxyphosphate cement for 1 week. B—dentin, C—pulp, D—silver nitrate precipitate.

Fig. 6b. Tooth from same dog as *fig. 6a*. Cavity had been prepared and filled with zinc oxyphosphate cement for 1 week prior to application of silver nitrate solution and then left for 1 week before extraction.

Fig. 7. A—cavity, B—dentin, C—pulp, D—silver nitrate precipitate in dentinal tubules in communication with pulp, E—silver nitrate precipitate in dentinal tubules in communication with cavity but not pulp.

Fig. 8. Ground section of human upper right second incisor. A—cavity, B—dentin, C—pulp chamber, D—degenerated dentin and silver nitrate precipitate.

place— , the penetration was less marked in the control teeth. *Fig. 6a* shows the penetration of silver nitrate into the dentin from a cavity to which the solution had been applied immediately after preparation. Penetration into the dentin from a cavity which had been filled with zinc oxyphosphate cement for

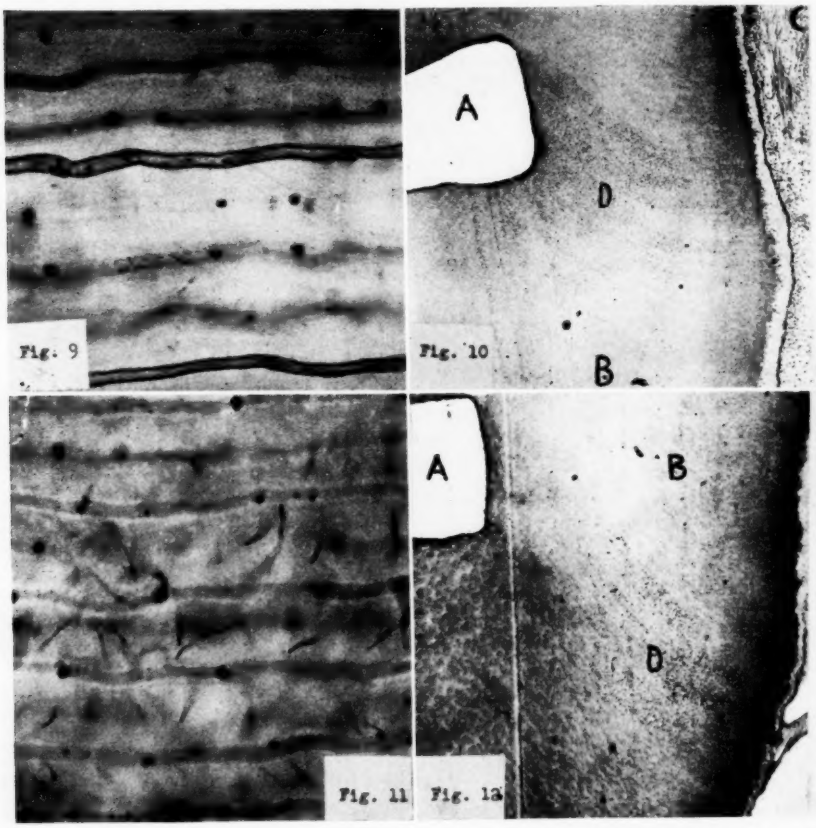

Fig. 9. High magnification of dentinal tubules underneath cavity in *fig. 11* showing silver nitrate precipitated particles in dentinal tubules.

Fig. 10. Decalcified section of human upper left second incisor. A—cavity, B—dentin, C—pulp, D—silver nitrate precipitate.

Fig. 11. High magnification of dentinal tubules in area D of *fig. 10*, showing silver nitrate precipitate in dentinal tubules and their lateral branches.

Fig. 12. Photomicrograph of pulpless tooth showing the penetration of the silver nitrate solution which had been applied to cavity A, B—dentin, C—pulp chamber, D—silver nitrate precipitate.

one week before the silver nitrate application, is illustrated by *fig. 6b*. Both teeth are from the same animal and the same amount of time elapsed in each between the time of application of the solution and the removal of the teeth.

It was observed that the silver nitrate solution penetrated also into dentinal tubules that were not in communication with the pulp but were in communication

with the cavity; in other words into that dentin which was undermined by cutting off the pulpal end of the tubules (*fig. 7*).

C. Silver Nitrate Experiments on Human Teeth

The same degree of penetration of silver nitrate into the dentin was observed in decalcified sections as ground sections. Ground sections from a human upper right second incisor are represented in *figs. 8* and *9*. The cavity was prepared and filled 24 hours before the silver nitrate solution was applied, and the tooth was extracted one week later. In *fig. 8* the cavity preparation is seen at (A), silver nitrate penetration at (D). At this magnification one cannot clearly distinguish between the silver precipitate, the opaque dentin, or gas in the dentinal tubules. However, at a high magnification, *fig. 9*, the silver precipitate can be clearly distinguished in the dentinal tubules. *Figs. 10* and *11* are from the upper left second incisor of the same individual. This tooth was decalcified before sectioning. The cavity (A) was also prepared and filled 24 hours before the silver nitrate solution was applied, and the tooth was extracted 1 week later. *Fig. 10* shows the penetration of silver nitrate (D). A higher magnification of the tubules in this area can be seen in *fig. 11*. The silver nitrate precipitate can be seen in the dentinal tubules and appears the same as that in *fig. 9* which is a ground section. The precipitate can also be observed in the side branches of the tubules.

Silver nitrate penetrated readily through the dentin of pulpless teeth. *Fig. 12* shows a cavity (A) in a pulpless tooth; silver nitrate has penetrated through the dentin and into the pulp chamber.

DISCUSSION

The results of our experiments indicate that the penetration of silver nitrate solution into dentin is a phenomenon which is not necessarily associated with the flow of a tissue fluid. The appearance of the so-called "calcific barrier" and the irregular dentin adjacent to the injured dentinal tubules as pictured in *fig. 3* should obviously interfere with the normal flow of tissue fluid. The fact that the silver nitrate precipitate reached through the so-called barrier and irregular dentin into the pulp (*figs. 3, 4, 5*) demonstrates that the dental lymph is not responsible in this instance for the transportation of the precipitate particles. The fact that the silver nitrate penetrated through the dentin of pulpless teeth (*fig. 12*) and the fact that silver nitrate penetrated into tubules which were not in communication with the pulp (*fig. 7*) further indicates that living processes are not an essential factor in the penetration of silver nitrate. Such phenomena as capillary attraction, diffusion and differences in pressures may explain the penetration.

In none of our sections was there any evidence of the silver precipitate being redistributed into the dentinal tubules, once it had reached the pulp. This applies to sections of teeth to which silver nitrate had been applied immediately following cavity preparation before any visible changes had occurred in the pulp and in the dentin. It also applies to those teeth which had undergone changes of both the dentin and the pulp.

SUMMARY

An investigation was undertaken to determine whether there is any association between the penetration of silver nitrate solution into dentin and the flow of a tissue fluid (dental lymph).

Degenerative changes in the dentin were produced experimentally by cavity preparation and fillings in dog and human teeth. Silver nitrate solution was applied to teeth which had undergone dentin degeneration, irregular dentin formation, and pulp injury. As a control silver nitrate solution was applied to dentin which had not undergone any visible changes, and to dentin of pulpless teeth. The penetration of the product of silver nitrate precipitation into degenerated dentin, through the so-called "calcific barrier" into irregular dentin, and into the pulp, was compared with the penetration into sound dentin and dentin of pulpless teeth. There were no appreciable differences. The findings were identical in both ground and decalcified sections.

CONCLUSIONS

1. The so-called "dead tracts," "metamorphosed dentin," "calcific barrier," and irregular dentin are penetrable by silver nitrate solutions.

2. There is no appreciable difference in the penetration of silver nitrate solution into dentin which has undergone degenerative changes as compared to the penetration into sound dentin and dentin of pulpless teeth. The penetration of silver nitrate solution into dentin is a phenomenon not necessarily associated with the flow of a tissue fluid.

3. The penetration of silver nitrate solution cannot be used as an indicator of dentin metabolism.

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A SURVEY OF THE INCIDENCE OF DENTAL CARIES IN THE RHESUS MONKEY[1]

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The rhesus monkey (*Macaca mulatta*) has the same deciduous and permanent dentitional formula as man. If a regimen could be devised to increase its susceptibility to tooth decay, the rhesus monkey would be a valuable laboratory animal for dental caries research. Granados ('44) described the type of lesions occurring in the molar teeth of 8 three-year old monkeys and 1 nine-year old monkey. Schultz ('35) made an extensive survey of the incidence of tooth decay in several species of primates. In 252 skulls of wild *Pithecus rhesus* (*Macaca mulatta*), the per cent of animals with carious teeth was: 0 in the young, 2.1 in the adults and 13.7 in the aged. In 290 skulls from captive monkeys of the same species, he observed that the per cent frequency of animals with carious teeth was higher: 1.3, 9.7, and 23.1, respectively. Anderson and Arnim ('37) observed carious lesions clinically similar to those found in man in 3 of 76 rhesus monkeys. Knap ('41) stated that caries occurred, in some degree, in about 4% of over 500 skulls of Netherlands-Indies monkeys. However, in *Macacus rhesus* (*Macaca mulatta*), he observed that caries could not be induced by specific diets: well cooked food, much sugar, much salt, nor by exclusion of sunlight. These reports indicate that the susceptibility of the rhesus monkey to tooth decay is too low to allow it to be used extensively in dental caries research. However, a survey of the caries incidence in 138 skulls of rhesus monkeys which had been maintained in this laboratory for varying periods indicates that the frequency of caries incidence under certain conditions may be much higher than previously reported.

PROCEDURE AND RESULTS

The skulls of the rhesus monkeys studied in this survey were obtained at the termination of experiments designed to study the nutrition of the monkey and the relation of nutrition to the incidence of experimental poliomyelitis. In the course of these experiments, the monkeys were maintained for periods varying

[1] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. This work was supported in part by a grant from the Nutrition Foundation, Inc., New York. Many of the monkeys used in this survey had been maintained on experiments of a joint project with the Department of Medical Bacteriology supported by a grant from the National Foundation for Infantile Paralysis, Inc. Received for publication Apr. 5, 1945.

We wish to express our appreciation to Anna Lou Hansen for technical assistance in this study.

from a few days to 3 years on various nutritionally adequate and deficient modifications of the basal ration described by Waisman ('43). This ration was composed of: sucrose 73%, casein 18%, corn oil 3%, cod liver oil 2% and salts IV (Phillips and Hart, '35) 4%. This ration was supplemented with ascorbic acid, the crystalline members of the B complex and various liver preparations, except where 1 or more of the nutritionally required factors were omitted in order to study that deficiency or combination of deficiencies. In some of the vitamin deficiencies studied, particularly in ascorbic acid deficiency, there appeared to be an earlier and higher incidence of carious lesions than in the monkeys which received a complete ration. However, there were not enough examples of any single deficiency to warrant definite conclusions without further extensive study with larger groups of monkeys. In this survey the incidence of carious lesions will be reported in relation to the length of the experimental period.

Early in this investigation, it was observed that the evaluation of the incidence of carious lesions could not be based solely upon the observation of the surfaces of the teeth. Even when the surfaces were studied at a magnification of 30x with the aid of a binocular dissecting microscope, it was very difficult to determine whether or not some of the developmental fissures and stains were carious (*figs. 1* and *2*). In almost all cases, the tine of an explorer was much too wide for the investigation of the suspicious regions. Therefore, examinations were made with a No. 42 smooth, dental broach. The diameter of this instrument was small enough to be used to probe all suspicious fissures. Regions were recorded where obvious carious lesions existed and where suspicious areas were observed.

The only satisfactory method found for the evaluation of the incidence and extent of tooth decay was a progressive grinding and observation of many successive parallel planes of the tooth. It was easiest to grind the upper quadrants and then the lower ones. In both cases the plane of grinding was parallel to the occlusal surface of the teeth. Thus all developmental fissures and stains could be followed to any desired depth from the occlusal surface. Wherever more detailed examination of the lesion was desired, ground sections were made of the area in the appropriate plane. No area was called a carious lesion in this survey unless there was a definite area of carious enamel with some evidence of penetration into the dentin.

A summary of the caries incidence in these skulls is presented in Table I. The regions of tooth decay which could be seen by inspection of the surfaces of the teeth have been recorded in the table as "obvious carious lesions." The total or actual incidence was determined by observations in the gross plus the ground preparations and was recorded as "carious lesions." It can be readily seen that both the per cent of monkeys with obvious carious lesions and the per cent of monkeys with carious lesions increased with age or the length of time the monkeys were on experiment. For each time interval, the per cent of monkeys with carious lesions is considerably greater than the per cent of monkeys with obvious dental caries, except in the first period.

The average number of carious lesions and the average number of carious teeth

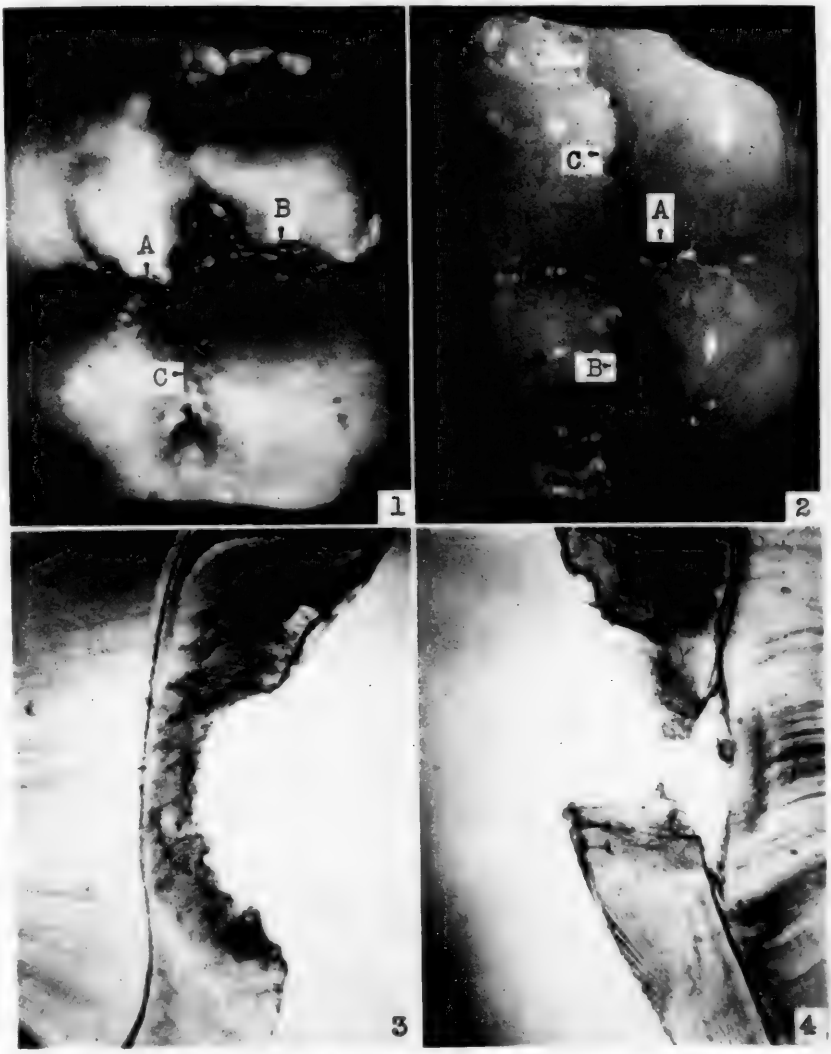

Fig. 1. Occlusal surface of a first permanent molar. Inspection of this surface shows chipping about fissures at A and B. No definite signs of decay could be found. Grinding revealed definite carious lesion with exposure of dentin at A, B, and also at C, where no evidence was visible from occlusal surface. Dark stain in the central sulcus was hard and non-carious. ×10 (orig. mag.).

Fig. 2. Occlusal surface of a first permanent molar. Obvious carious lesion is seen at A and a smaller one at B. Grinding exposed another at C. ×10 (orig. mag.).

Fig. 3. Ground section of a first permanent molar. Wide, carious lesion of enamel of mesial, proximal surface is shown. ×60 (orig. mag.).

Fig. 4. Ground section of a first permanent molar. Carious lesion with dentin exposure is seen on mesial, proximal surface. ×60 (orig. mag.).

were calculated on the number of monkeys with carious teeth and not on the total number of monkeys in the group. The average number of carious lesions varied from 3.3 per monkey in the 1.5 to 3 month group to 6.8 in the 24–36 month group. The average number of carious teeth increased from 2.2 to 4.8 in the same groups.

There were slightly more carious teeth in the upper jaws than in the lower. The number of carious lesions was considerably higher in the upper jaws than in the lower. This is the opposite relation to that observed in the rhesus monkey by Knap ('41) and in man by Klein and Palmer ('41). However, Schultz ('35) observed that in both the catarrhines, which include the rhesus monkey and the platyrrhines, caries was somewhat more frequent in the upper than in the lower jaw. A definite, bilaterally symmetrical distribution of carious lesions was observed. Bertram and Brown ('43) and others have reported a similar phenomenon in man.

TABLE I

A survey of the frequency of caries incidence in 138 rhesus monkeys

NO. OF MONTHS ON EXPERIMENT (RANGE)	NO. OF MONKEYS	NO. WITH OBVIOUS CARIOUS LESIONS	TOTAL NO. WITH CARIOUS LESIONS	% WITH OBVIOUS CARIOUS LESIONS	TOTAL % WITH CARIOUS LESIONS
0–1.5	11	0	0	0	0
1.5–3	33	2	6	6	18
3–6	31	3	8	10	26
6–9	13	3	5	23	38
9–12	9	1	4	11	44
13–15	5	1	3	20	60
15–18	11	5	6	45	55
18–21	12	6	8	50	67
21–24	4	2	3	50	75
24–36	9	7	8	78	89

When these monkeys were placed on the experimental regimen, they all had complete deciduous dentitions. The first permanent molars were fully erupted in about 80% of the monkeys and were in the process of eruption in almost all of the remaining animals. Two or 4 permanent incisors were in eruption in an occasional monkey when the experimental regimen was begun. At the end of the first year, these monkeys had from 2 to 8 permanent incisors erupted or in the process of eruption but the second permanent molars were not erupting in any of the animals. By the end of the second year, all the permanent incisors were completely or partially erupted and the lower second permanent molars were partially erupted in half of the monkeys. Those monkeys which were on these experiments for 3 years had completely erupted incisors, completely erupted second molars, and partially or completely erupted canines and bicuspids. In one of these monkeys, the lower third permanent molars were partially erupted.

The developmental fissures between the cusps of the first permanent molars were the most common sites of carious lesions. The fissures of the second per-

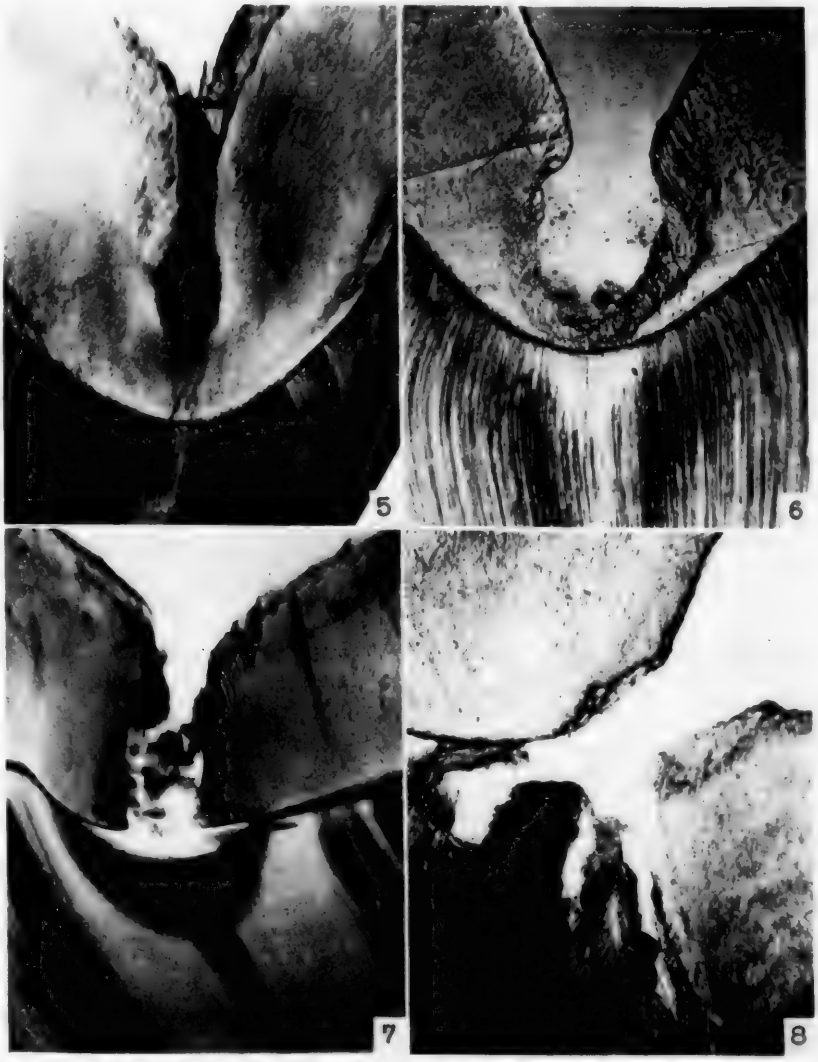

Fig. 5. Ground section of a first permanent molar. Normal fissure is seen filled with debris between distal cusps. ×60 (orig. mag.).

Fig. 6. Ground section of a first permanent molar. Fissure is shown with early carious development of enamel and slight penetration of dentin. ×60 (orig. mag.).

Fig. 7. Ground section of a first permanent molar. Carious lesion in this case has exposed dentin and development of lesion along dentino-enamel junction is occurrring. Note very narrow opening to occlusal surface and intact enamel at surface. ×60 (orig. mag.).

Fig. 8. Ground section of a first permanent molar. Widespread destruction of dentin has occurred with undermining of enamel. Enamel at edge of lesion is apparently normal with no staining. ×60 (orig. mag.).

manent molars were likewise very susceptible to tooth decay but the incidence was not as high, which was due, no doubt, to the shorter exposure to the experimental conditions. These fissures varied greatly in width and depth. In all cases, the normal fissure was sufficiently narrow that it could not be explored by a No. 42 smooth, dental broach. However, these fissures were commonly filled with debris (*fig. 5*). The actual condition of these fissures could not be determined from a surface inspection. It was necessary to grind the crown of the molar until the bottom of these fissures had been exposed. Frequently carious lesions were found which had progressed very extensively in the enamel at the base of the fissure and into the dentin without enough destruction of the enamel at the occlusal surface to permit detection. In other cases, the sides of the fissure appeared to be chipped at the occlusal surface but no decay was obvious (*fig. 1*). In most of these, grinding revealed a carious lesion of the type shown in *fig. 6*. These types of lesions progressed very rapidly after the dentin had been penetrated. The path of most rapid carious activity was along the dentino-enamel junction (*figs. 7* and *8*).

Carious lesions were also found in the sulci of the tooth surfaces. The most common sulci involved were the narrowest and deepest ones. The least common regions of tooth decay were the incisal surfaces of the incisors and the proximal surfaces of the molars. When a proximal surface was carious, a much wider area of enamel was affected than in the other regions of caries formation (*figs. 3* and *4*).

Carious lesions were rarely observed in the deciduous teeth. The most common regions affected were the proximal surfaces. A few lesions were found in the fissures between the cusps of the molars.

In all the regions of tooth decay observed, the enamel appeared to have a very high resistance to the processes of decay. *Figs. 6*, *7* and *8* show the type of narrow lesion that is present in the enamel. However, decay progresses rapidly in the underlying dentin. It appeared that once the dentin had been penetrated the spread of the carious lesion in the enamel was negligible. The enamel about regions of obvious carious lesions such as A and B in *fig. 2* was very hard. Extension of lesions A and B in size appeared to be by chipping and fracturing of the undermined enamel rather than by any continued carious extension in the enamel.

DISCUSSION

We have observed a higher frequency of dental caries incidence, in the rhesus monkey than has been reported previously. Anderson and Arnim ('37) and Knap ('41) did not state what criterion they used to distinguish a carious lesion. Schultz ('35) stated that he called a tooth "carious" if it contained a more or less irregular cavity, caused by the progressive destruction of dental tissue. When the definition of Schultz ('35) was applied in the present survey, the frequency of caries incidence in the first year on experiment was found to be very low, but became rapidly higher during the second and third years of the experimental period. However, in all cases the frequency of caries incidence, as determined by

inspection of parallel planes of ground sections, was higher than that observed by Schultz in wild and captive rhesus monkeys of a similar age range. By both methods of evaluation it appears that the rhesus monkey is more susceptible to tooth decay on the regimen described than when fed rations composed of natural foodstuffs.

In the course of this survey no skull was examined in which the carious lesions were as numerous or as rapidly progressive as in rampant caries in man. However, all the carious lesions observed appeared similar to the more slowly progressive caries found in man. The only striking difference observed was the resistance of the enamel to the processes of decay as evidenced by the small initial lesion through the enamel. The dentin appeared to be much less resistant than the enamel since the lesions spread very rapidly in the dentin, particularly along the dentino-enamel junction.

SUMMARY

A survey of the frequency of dental caries incidence in 138 rhesus monkey skulls is reported. The incidence of obvious carious lesions was determined by observation of the intact surfaces of the teeth. The frequency of caries incidence observed was higher than has been reported previously. The monkeys had been maintained for periods varying from a few days to 3 years on various nutritionally adequate and deficient rations. The frequency of caries incidence increased with the length of time the monkeys were maintained on these dietary regimens.

In order to detect all the carious lesions which were present, it was shown that the teeth must be examined by grinding in order to observe successive planes parallel to the occlusal surface. Only in this way can all the lesions at the base of the sulci and in the developmental fissures be seen and evaluated. In each group of monkeys the frequency of caries incidence, when determined by this method, was higher than when determined by inspection of the intact surfaces of the teeth.

The carious lesions observed in the rhesus monkey are very similar to those found in man. A definite, bilaterally symmetrical distribution was found. However, the caries incidence in the upper was higher than in the lower jaw.

The susceptibility of the rhesus monkey to tooth decay observed in this survey is sufficiently high to warrant the extensive use of this species in dental caries research. The high susceptibility reported is apparently due to the type of dietary regimen. It would appear from the data at hand that diets which contained high amounts of sucrose produced a greater frequency of dental caries in the monkey than natural rations. Thus, the rôle of nutrition assumes a vital relation in the production of or prevention of dental caries. However, a period of at least 2 years is necessary for the satisfactory assay of the effect of any causative or preventive treatment upon the frequency of caries incidence in the monkey.

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INFLUENCE ON THE RAT INCISOR OF VITAMIN K DEFICIENCY IN THE PRESENCE OF SULFADIAZINE CONTAINING DIETS[1]

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While marked vitamin K deficiency can be developed in rats simply by omitting the vitamin from the diet (1), the disease is more satisfactorily produced when vitamin K production by the intestinal flora is prevented. Incorporation in the diet of 1% sulfadiazine has been found effective for this purpose (2). The present study, originally designed to determine whether vitamin K, like vitamin E (3, 4, 5, 6), influences enamel pigmentation in the rat incisor, was carried out with sulfadiazine-containing diets.

EXPERIMENTAL

Three groups of albino rats, each comprising 6 newly-weaned animals, were reared for 91 days on the following diets: Group I (K-deficient, sulfadiazine) received sucrose 60%, alcohol-extracted casein 20%, ether-extracted yeast 10%, salts U. S. P. No. 2 5%, cod liver oil 5%, sulfadiazine 1%, and d,1-alpha tocopherol acetate 10 mg. percent; II (vitamin K, sulfadiazine), same as I plus 1 mg. percent of tetrasodium salt of 2-methyl-1,4-naphthoquinone diphosphoric acid; and III (normal control), dog chow. Body weights and incisor eruption, attrition, and pigmentation were recorded weekly in all animals.

RESULTS AND DISCUSSION

No significant differences were observed between animals on sulfadiazine-containing diets (I, II) in growth, enamel pigmentation, and rates of eruption and attrition of incisor teeth, irrespective of vitamin K status. There was, however, a striking deficiency of growth and a decline of eruption and attrition rates of incisors in rats on sulfadiazine-containing rations (I, II) as compared with control animals (III). These differences are indicated in *figs. 1* and *2*.

All animals receiving sulfadiazine suffered from severe intoxication manifested by general weakness, anemia, and early hematurea. Three rats in group I and 1 in II died between the 26th and 71st day of the experiment. At the close of the experimental period the prothrombin time (7) was 110–360 seconds in K-deficient animals (I) and about 33 seconds in K-sufficient rats (II). Group I animals

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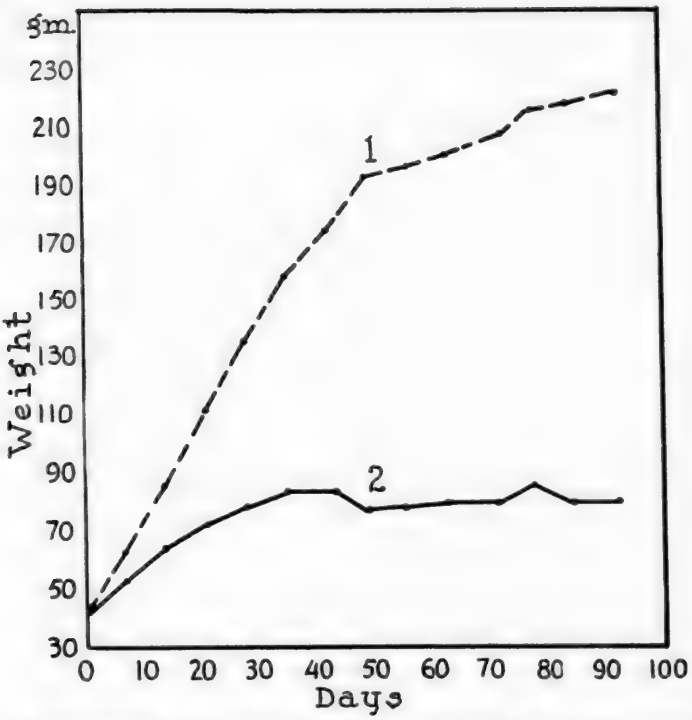

Fig. 1. Retarded growth of rats receiving diets containing 1% of sulfadiazine (2) as compared with that of control animals (1). Vitamin K deficiency had no appreciable effect.

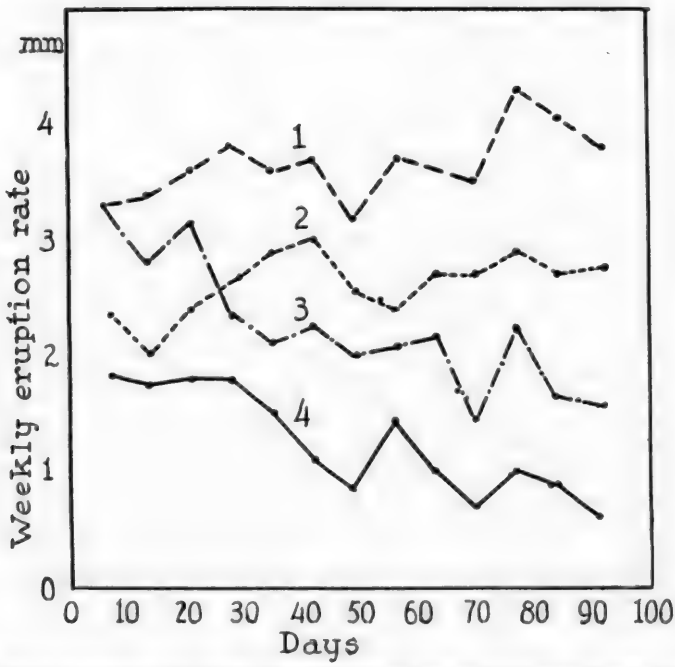

Fig. 2. Eruption of mandibular and maxillary incisor teeth of control animals (1, 2) and animals receiving diets containing 1% of sulfadiazine (3, 4).

developed hemorrhages subcutaneously and in the joints, lungs, and kidneys. At autopsy calculi were found in the bladders of all animals which received sulfadiazine. These rats also developed scaly tail.

Lack of differentiation between K-deficient (I) and K-sufficient (II) rats would indicate that vitamin K itself probably does not influence enamel pigmentation nor eruption and attrition of incisor teeth. Observed differences between animals fed sulfadiazine (I, II) and those on a sulfadiazine-free diet (III) signify that sulfadiazine given to prevent vitamin K formation in the intestine causes severe intoxication and retardation of growth reflected in lowering of eruption and attrition rates of the incisor teeth.

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LACK OF CORRELATION BETWEEN DENTAL CARIES AND SALIVARY AMYLASE[1]

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A high positive correlation between salivary amylase and dental caries has been reported (1–3). Others have not found this (4). To test the relation, we examined groups of medical and dental students who were determining their salivary amylase activity by a standard method (5), paraffin chewing being used

TABLE I

Caries experience (DMF) vs. amylase activity

DMF	RANGE OF AMYLASE UNITS	AVERAGE NUMBER AMYLASE UNITS	NUMBER OF STUDENTS IN GROUP
0– 5	50–200	93	9
6–10	25–125	81	11
11–15	47–143	80	19
16–20	50–125	75	7
Total			46

as a method of stimulation. Three groups were examined in a routine manner by dentists.

The students in the first group of over 100 were classified as having "high," "moderate," or "low" degrees of caries on the basis of the number of open and filled cavities. No correlation between caries experience and amylase was found. In examination of the second group of 46 students, caries experience was recorded by giving each decayed, missing or filled tooth a count of 1 (D.M.F. index). The lack of correlation between caries experience and amylase is shown in Table I. Since no correlation was found when filled teeth were included, only open lesions (caries activity) were recorded in the examination of the third group of 105 students with results shown in Table II.

CONCLUSION

The relation between dental caries and salivary amylase was studied in 250 individuals, mostly young men. No correlation was found.

[1] Received for publication May 21, 1945.

TABLE II

Open carious lesions vs. amylase activity

OPEN CARIOUS LESIONS	RANGE OF AMYLASE UNITS	AVERAGE NUMBER AMYLASE UNITS	NUMBER OF STUDENTS IN GROUP
	Examiner 1		
0	59–125	86	6
1	23–111	71	5
2	33– 95	61	4
3	69–470	146	8
4	25–400	104	9
5	83–120	101	2
6	45– 80	58	6
7	72–100	86	2
11 or over	25–100	58	3
Total			45
OPEN CARIOUS LESIONS	RANGE OF AMYLASE UNITS	AVERAGE NUMBER AMYLASE UNITS	NUMBER OF STUDENTS IN GROUP
	Examiner 2		
0	46–200	78	26
1	17–420	135	16
2	50–117	85	7
3	30–100	56	7
6	40– 63	52	2
9 and 10	40– 63	53	2
Total			60

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FORMIC ACID-SODIUM CITRATE DECALCIFICATION AND BUTYL ALCOHOL DEHYDRATION OF TEETH AND BONES FOR SECTIONING IN PARAFFIN[1]

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Oral calcified tissue, which includes extracted teeth, teeth in situ, and alveolar bone, is usually prepared for histological examination by the general method of nitric acid decalcification, ethyl alcohol dehydration, and celloidin embedding. This paper describes a different method of tissue preparation which consists of formic acid-sodium citrate decalcification, butyl alcohol dehydration, and paraffin or celloidin embedding. The technic, described here in detail, was arrived at after 10 years of experimentation. During the last 3 years of this period, approximately 500 pieces of tissue were prepared and from these some 30,000 slides. An analysis of the various problems involved in the preparation of oral calcified tissue are presented in an effort to demonstrate the advantages of the proposed technic of preparation.

For purposes of clarity, the outline of the technic is followed by an analysis of each step of the method in sequence.

METHOD

1. Fix in 10% aqueous formaldehyde solution.
2. Wash in running tap water or several changes of distilled water for 2–5 hours.
3. Decalcify in formic acid-sodium citrate reagent until chemical test is negative for the presence of calcium.

Formic acid-sodium citrate reagent:

Solution A: 90% formic acid C.P.	1 part
Distilled water	1 part
Solution B: Sodium citrate C.P.	20 grams
Distilled water	100 c.c.

Just before use combine equal portions of Solution A and Solution B. Use large volumes of the reagent and change daily until test is negative for calcium.

Test: To 5 c.c. of the used decalcifying reagent, add 1 c.c. of concentrated ammonium hydroxide, mix thoroughly, then add 0.1 c.c. of a saturated aqueous solution of ammonium oxalate. A precipitate will form when calcium is present. Repeat the additions of 0.1 c.c. amounts of ammonium oxalate at intervals of 15–20 minutes until a total of 0.4 c.c. has been added. If a precipitate fails to form after the addition of 0.4 c.c. of ammonium oxalate, leave the tissue in the same decalcifying reagent for at least 48 hours longer and repeat the test. When the test remains negative for 3 days in the case of a single tooth and for 1 week in the case of larger tissues, proceed with dehydration and infiltration.

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4. Dehydrate and infiltrate as follows:
   A. For paraffin embedding:
      1. Wash in running tap water or several changes of distilled water for 5–24 hours.
      2. 30%, 50%, 70% ethyl alcohol each for at least 24 hours.
      3. Solutions #1, 2, 3, 4, 5, and 6 of butyl alcohol (see Table I) each for at least 48 hours.
      4. Infiltrate with paraffin of 56°–58°C. melting point for 5–24 hours.
   B. For celloidin embedding:
      1. Wash in running tap water or several changes of distilled water for 5–24 hours.
      2. 30%, 50%, 70%, 80%, 95% ethyl alcohol, and equal portions of 95% ethyl alcohol and ether, each for at least 24 hours.
      3. Infiltrate with 2%, 4%, 6%, 10%, 14%, and 25% celloidin for at least one week in each.

Rapid fixation of all tissue elements, which are of paramount importance in critical histological studies, is difficult when calcified oral tissues are involved because the penetration of the fixing fluid through dense alveolar bone, enamel, and dentin is a slow process. Post-mortem changes occur in the center of the specimen before the fixing agent can penetrate the periphery. Perhaps, the most seriously affected tissue of this type is the pulp.

Many methods have been proposed to facilitate the penetration of fixing agents into the pulpal tissue. Cutting of holes or slots, and stripping of the dentin are some of these practices. Hill (1) believed "satisfactory fixation of dental pulps" is obtained "by immediate grinding of the side of the tooth under water until the coronal pulp was freely exposed" and then placing it in the fixative. Willman (2) advocated cutting holes in the side of the tooth or cutting off the apex. Cowdry (3), on the other hand, says that these practices are "apt to disturb the position of the pulp and should be avoided." One undesirable result is the disturbance due to the trauma and heat subjacent to the cut. Certainly, the reduction of the depth through which the fixing fluid has to pass will reduce the time involved. However, there is some doubt in the author's mind whether the so-called fixation artifacts are eliminated by these methods.

The problem of excellent, rapid, cytological fixation of oral calcified tissues is still unsolved. It must be noted, also, that subsequent treatment can influence profoundly the primary fixation image.

Choice of a fixing reagent is dependent upon the tissue itself and the purpose for which it is to be preserved. Special reagents prepare the tissue for study of specified elements or reactions and chemically prepare for the application of selective stains. Formaldehyde solution is an excellent general fixing reagent and permits the subsequent use of a large variety of staining methods. Many questions may be raised as to the advisability of its use. However, it is an agent which may be handled by anyone not acquainted with the details of the special agents. Its use in our laboratory is due to the fact that most of the tissues come from other departments or from the outside and take varying lengths of time to reach the laboratory. Under these circumstances, the use of special reagents requiring a few hours or days for fixation is precluded. Furthermore, autopsy material has already been subjected to the influence of formaldehyde in the em-

balming fluids. Tissues may be left in formaldehyde solution indefinitely although there is some indication that after a year the stainability of the tissue is decreased.

Following fixation, tissues should be washed in running water in order to remove accumulations of debris and as much of the fixing fluid as possible. If there is any danger of diatoms, rust, etc. in the water supply or if the tap water is chemically impure, it is preferable to wash in several changes of distilled water.

Decalcification is most important in the preparation of oral calcified tissues for microscopic examination. It is important from two standpoints: first, sections of teeth, bone, and surrounding tissues are difficult to obtain without removal of the calcium; and second, the effect of the various chemical decalcifiers upon the tissue components differ.

This second important item is the greatest problem of the whole technic. The primary fixation picture can be changed by subsequent treatment. Sections must be carefully studied so that correct conclusions are drawn as to the effect of the decalcifier upon the cellular elements. Distortion and macerations can be demonstrated by the use of chemicals following fixation. Grossly, the tissue may appear unchanged but closer study reveals that the cellular elements exhibit swelling, shrinkage, vacuolization, disruption, and fraying not attributable to pathological conditions. Short periods of action by the decalcifier upon the tissue do not necessarily result in these changes. However, continued action to bring about complete decalcification very often results in distortion and actual destruction of the tissue. This is the reason that small pieces of tissue measuring 2 mm. x 2 cm. x 2 cm. are recommended when decalcification is necessary. Single teeth are larger and pieces of tissue containing up to 6 teeth are much larger and must remain in the decalcifier for long periods of time. Therefore, the results of the longer action must be evaluated carefully before any decalcifier is used upon oral tissue for histological examination.

Nitric acid is unfortunately the most common decalcifying reagent used to decalcify oral tissues. It is used in aqueous (2, 4, 5, 6, 7, 8) or alcoholic solution, with or without the addition of a preservative such as phloroglucin. Small pieces of tissue can be decalcified quickly and well in 5% to 10% aqueous nitric acid. Single teeth decalcify fairly well but larger specimens exhibit many distortions and destructions. In order to remove the calcium from the center of a piece of tissue, the periphery is subjected to the action of the acid for too long a period (over-decalcification). Maceration of the exterior is common and distortion takes place for some distance into the specimen on all sides. The stainability is markedly decreased with a resultant diffuse eosin stain. For these reasons, nitric acid as a decalcifying reagent is undesirable for blocks of tissue larger than 2 mm. x 2 cm. x 2 cm. When the tissue is thin in at least one dimension, the acid penetrates quickly and the decalcification is complete before too much harm is done. Cytological studies on any nitric acid decalcified material are open to a great deal of question.

Inorganic acids, other than nitric, have been used for decalcifying agents, among which are hydrochloric, sulphurous, and sulfuric acids. Each has a place

in decalcification under certain circumstances, but they have been rejected for use on human oral tissue for varying reasons. Of the organic acids, acetic and trichloracetic acids are those most commonly used, and, although decalcification is rapid and the preservation good, swelling occurs. Jaffé (9) states that "in general, it may be said that mineral acids possess greater decalcifying power, but are most injurious to the nuclei, while organic acids produce the greatest swelling of the fibrils [of bone] but less injury to the nuclei, if exposure is not too prolonged."

Some time ago, in the search for a decalcifying fluid which could be employed upon oral tissue of all types and sizes and would eliminate or diminish distortion we were introduced by the Department of Pathology at Tufts College Medical School to a reagent made up of equal portions of 45% aqueous formic acid and 20% aqueous sodium citrate, which was being used upon bone with excellent preservation of the cellular elements. The original source of the reagent is unknown. The preliminary results of the use of the mixture upon teeth and bone substantiated the observations upon bone as to the cellular preservation. This brought about the continuation of its employment and observations of the action of the reagent upon tissues from the oral structures.

Formic acid has been used as a decalcifying agent in oral histology and pathology usually as a strong aqueous solution of 33% or combined with formol. In 1930, Evans and Krajian (10) introduced "a new method of decalcification" which was a combination of equal parts of 85% aqueous formic acid and 20% aqueous sodium citrate. Glickman and Wood (11) are the first to report the use of our reagent (45% aqueous formic acid and 20% aqueous sodium citrate) upon oral calcified tissue. Lillie (12) has studied combinations of formic acid and sodium citrate in relation to the successful staining of bone marrow.

The objection to the use of formic acid is the swelling action it has upon tissues. Jaffé (9) said that it causes great swelling of the collagenous fibers. Evans and Krajian (10) stated that with their solution "the most prominent point is that the cellular elements are practically unaffected and take the stain apparently as perfectly as tissues that are not subjected to decalcification." These authors suggested that the citrate counteracts the swelling tendency of the formic acid.

In order to study more fully the effect of the combinations of formic acid and sodium citrate upon extracted teeth, normal teeth from the same patient were fixed simultaneously in 10% formaldehyde. Then one half of the number of the teeth were decalcified in equal portions of 85% formic acid and 20% sodium citrate; the other half were decalcified in equal portions of 45% formic acid and 20% sodium citrate. During the decalcification period, the teeth in the former reagent gradually became more glassy. Following dehydration and embedding in paraffin according to the method proposed in the article, it was found that the first group was very difficult to section. The second group sectioned easily.

A second series of studies were instituted to learn the effect of the components of the 45% formic acid and 20% sodium citrate reagent upon the cellular elements of normal tissue. Pieces of the same kidney and liver of a normal rat following fixation in 10% formol were subjected to the influence of the following

solutions: (1) 45% aqueous formic acid, (2) 20% aqueous sodium citrate, and (3) equal portions of 45% formic acid and 20% sodium citrate. The remainder of the technic was identical with the proposed method. The material was allowed to remain in the various solutions for 7 days.

The results of this study brought to light some very interesting facts. Those tissues passed through the formic acid alone exhibited great swelling of all tissue elements so that the structure of the organ was barely discernible. The nuclei were very poorly stained. The tissues passed through sodium citrate alone showed shrinkage of the cytoplasm throughout with excellent staining of the nuclei. Tissues run through the combination showed only slight swelling of the cytoplasm and collagenous fibers and the nuclei stained more brilliantly than those of similar untreated material.

As a result of these preliminary tests, the combination of equal portions of 45% aqueous formic acid and 20% aqueous sodium citrate is now used routinely for decalcification. The solutions are kept separate until needed. A crystal of thymol added to the sodium citrate aids in keeping down bacterial and fungal growth. Tissues to be decalcified are placed in 10 times their volume of the reagent, which is changed every day until decalcification is complete.

Testing chemically for the end-point in decalcification is the only accurate method for determination of the complete removal of the calcium. The methods of needle-testing, flexibility, and x-ray have been, in our experience, extremely variable in results. Williams (4) used the production of ammonium phosphomolybdate as an indicator with nitric acid decalcification. The chemical test outlined in the method is that published by Arnim (13).

The description of the test is clear and its application simple. However, some annotations are needed for better interpretation of the results. Testing begins on the 2nd or 3rd day of decalcification when the addition of 0.1 c.c. of ammonium oxalate to the reagent plus the ammonia causes a heavy precipitate. The end-point is approaching when a precipitate fails to appear upon the addition of 0.3 c.c. of ammonium oxalate and only a slight precipitate upon the addition of the fourth 0.1 c.c. When no precipitate appears upon the addition of 0.4 c.c. of ammonium oxalate, the tissues must be left in the same fluid another 3 days and tested again. This interval of 3 days is necessary because the density of the dentin and bone impedes the penetration of the reagent into the middle of the specimen. Judicious use of slight negative pressure at this time is recommended. Longer action of the acid and the negative pressure may cause a precipitate upon the addition of only 0.1 c.c. of ammonium oxalate. Larger specimens of tissue are more apt to react in this fashion than single teeth. Experience with this chemical method enables one to evaluate the results.

Towards the end of the decalcifying period, the tooth or larger specimen should be trimmed in the plane of cutting. This is done with a razor blade and is limited by the structures desired in the section. Two results are obtained by this practice: 1, a flat surface is provided for easier embedding; and 2, a larger area is exposed to the further action of the decalcifier.

The length of time required to completely decalcify tissues naturally varies

with the size of the specimen. A single extracted tooth subjected to the action of the reagent requires about 10 days for complete decalcification. As the specimen increases in size from a single tooth to a block of 5 to 6 teeth in situ, the decalcifying period extends into weeks and months.

Single extracted teeth	7–24 days
One tooth with surrounding tissue	14–30 days
Two teeth in situ	30–40 days
3, 4, 5, 6 teeth	1–2 months
Rat mandible	1–2 days
Rat head in toto	3–5 days
Dog blocks of 2–3 teeth	2–3 months

The extraction of the calcium takes slightly longer with the formic acid-sodium citrate reagent than with some of the other agents, but the results are well worth the time involved. Apparently, a long continued subjection to the reagent is not detrimental to the cellular and histological elements of the specimen. Overdecalcification is almost entirely eliminated as a hazard. This is a distinct advantage in many ways but perhaps most advantageous in the flexibility of the technic. Laboratory hours and intervals of absence do not cause the loss of valuable material.

Immediately following the negative test for the presence of calcium, the tissue is washed in running water or several changes of distilled water for 5 to 24 hours. Usually, tissues are transferred from formic acid directly into alcohol in order to counteract swelling. However, the addition of the sodium citrate to the reagent results in an elimination of swelling during this step. Both methods, washing and direct transfer to alcohol, seem to have very little difference in their effect upon the subsequent histological picture. Washing for 5 to 24 hours eliminates from the tissue any reagent which would tend to interfere with the subsequent staining.

After washing, the schedule of dehydration proposed above is followed. The time noted for the first part of the series is "at least 24 hours" and for the second part is "at least 48 hours." Our experience has been that a single extracted tooth can be moved up in the series this fast, but no faster without poor dehydration of the pulp. The density of the dentin obstructs the equilibration of the 2 adjacent solutions in the substance of the tooth. We prefer to use still longer periods of time for each member of the series even for a single tooth and increase the time as the block increases in size.

Butyl alcohol as a dehydration-infiltration chemical has long been used in botanical technic and has only recently come into use in animal technic. Stiles (14) believed this is because there has been little experience with it. Lang (15) considered butyl alcohol the most satisfactory agent and least likely of all dehydration reagents to "exercise deleterious effects upon the primary fixation image." Stowell (16) recommended tertiary butyl alcohol in his TBEA series after Johansen as giving the "most satisfactory results in general micro-technic work both in ease of sectioning and in final microscopic quality of tissue."

The use of butyl alcohol dehydration in oral histological and pathological

technic is almost unknown. Fish (17) mentioned its use. Williams (4) noted that butyl alcohol followed by paraffin brought no success. However, she does not mention the series used or the technic followed. Gairns (18) said butyl alcohol used for clearing resulted in no noticeable difference.

There are 4 butyl alcohols which vary in their physical properties. Of these, n-butyl alcohol has the highest boiling point (118°) and the lowest solubility in water (8.3 gm. per 100 gm. of water); t-butyl alcohol has the lowest boiling point (83°) and is completely soluble in water. N-butyl alcohol is commonly used in botanical and animal technic. Combinations of n-butyl alcohol and ethyl alcohol in varying proportions were tried for dehydration of oral tissue and resulted in successful sectioning in paraffin. The following table shows the combinations now in use and referred to under the method.

TABLE I

	SOLUTION #					
	1	2	3	4	5	6
Distilled water (cc.)	30	15	—	—	—	—
95% ethyl alcohol (cc.)	50	50	45	25	—	—
N-butyl alcohol (cc.)	20	35	55	75	100	100

Johansen (19) has suggested that due to the low miscibility of n-butyl alcohol with water, it was unsatisfactory with many plant tissues because of dessication. Furthermore, t-butyl alcohol has all the requirements of a successful dehydrator as it is miscible in all proportions with water, ethyl alcohol, and paraffin and would replace all free water yet cause no alteration in the water-absorbing capacity of the tissue. Recently, a change was made to t-butyl alcohol instead of n-butyl in the dehydration series. This change has been made too recently to attempt, at present, any comparisons between t-butyl and n-butyl alcohol as dehydrators for oral tissues.

Dehydration of oral tissues should be much slower than with other tissues in order to prevent the separation of the organic components of different densities. Butyl alcohol permits slow dehydration without the serious hardening effect of ethyl alcohol. Hardening is negligible with butyl alcohol when compared with that obtained with ethyl alcohol. In addition, butyl alcohol is also a clearing agent which eliminates the necessity of clearing the tissues in other clearing agents which all have a tendency to further hardening effects.

One of the reasons for failures in the use of butyl alcohol is probably the fact that the infiltration period has been too short. Long periods of infiltration are absolutely necessary because of the slow miscibility of butyl alcohol and paraffin and the low volatility of the alcohol. The tissues may be placed directly in the melted paraffin or carried through a mixture of butyl alcohol and paraffin. In either case, the number of the changes of paraffin must be increased from the usual 2 or 3 to 5 or 6 with embedding in new paraffin. A 5-hour period of infiltration for a single tooth is the minimum. Longer periods of time are used

without the hardening experienced with the usual reagents. Single teeth section easily after 24 hours infiltration with paraffin of 56°–58° C. melting point and specimens as large as 3 teeth have been left in the oven for 3 days with excellent results. As Stiles (14) said, "it would seem that it is not the HOT paraffin which causes the serious hardening of tissues during infiltration but rather hot paraffin following certain types of dehydration and clearing."

A paraffin of high melting point should be used for bones and teeth. Soft tissues such as kidney and liver can be sectioned successfully at 5–6 micron using parawax as an embedding material. Hard tissues such as bones and teeth can be successfully sectioned only with a paraffin of 56°–58°C. melting point which gives a more solid matrix.

Subsequent to embedding, the block is trimmed to size and mounted for sectioning on a rotary microtome. Trim the block roughly to size and mount it on the cross-cut end of a rectangular piece of pine wood impregnated with paraffin. This mounted block is then placed in the carrier of the microtome and there accurately trimmed with 2 parallel sides by means of a Book block trimmer (20). The Book block trimmer is essentially a single edge razor blade set on a piece of wood or metal at an angle of 78°. This is the easiest trimmer to use and the simplest to construct of any that have been proposed. An added advantage is the resulting accurate alinement of the 2 surfaces of the block and the knife edge. With 2 motions, the block edges are parallel to the knife edge. Other block trimmers, more elaborate in construction, give parallel edged blocks but these are obtained away from the microtome. Then the block must be oriented in the carrier of the microtome so that the edges are parallel to the knife edge. At best this is a difficult manipulation. The Book trimmer accomplishes these 2 operations in 1, and much better than can be done manually.

Sectioning of the material is just as important to success with the technic as adherence to the schedule through the embedding. A few notes should be included on the sectioning procedure in order to aid in reproducing the results obtained.

First, the microtome knife, which is a very important tool, should be most carefully sharpened. Many excellent discussions on the technic of sharpening have been written so there is no need for repetition here. Reference may be made to Richards (21) and Bailey (22) for aid in obtaining the knife edge requirements for bone and teeth. These requirements are (1) an edge free from serrations at better than 100 diameters, and (2) a small bevel angle. The clamps for holding the knife in position should be placed as near to the paraffin block as allowable without interference with the block. All the set screws must be tightened to prevent any vibrations of the knife, carrier, or block during sectioning. The knife, when set in the microtome holder, is tilted until the angle between the surface of the block and the surface of the knife facet (the clearance angle) is that best suited for the material.

Second, the surface of the block should be exposed to water from time to time during cutting. After the initiation of the complete technic, difficulty was experienced in obtaining a good ribbon. All possible remedies, such as changing

the knife angle, critical embedding and blocking, resharpening of the knife, etc. were tried with no success. An article by David (23) in which she stated that the cut end of a block of bone should be exposed to water before cutting in order to obtain uniform sections gave a hint for remedy of the difficulty. Gairns (18) has recently suggested exposure to water of blocks of cat teeth and bone. Exposure of paraffin embedded teeth to water overnight resulted in good sectioning, but only to the depth to which the water had penetrated. Modifications followed these first experiments until at the present time the method used is as follows: cut the block until the desired orientation is obtained, then cover the surface of the block with absorbent cotton wet with cold water, remove the cotton and eliminate the excess water on the knife and block, cut about 15 sections, replace the wet cotton while these sections are mounted on slides, and repeat the process until the block has been sectioned. A little practice with the method enables one to determine the length of time necessary for the action of the water upon the next 15 sections. As we spread our sections upon hot water and then mount, the time involved in spreading, separation, and mounting of 15 sections is just about enough time for the softening action of the water upon the next set of sections.

The only limits to the use of paraffin as an embedding medium are not those imposed by the technic, but rather those imposed by the mechanical construction of the microtome. When tissues exceed in size a specimen containing 3 teeth in situ, the microtome knife cannot be made rigid enough to successfully cut them evenly. In addition, the microtome is so constructed that the strain of cutting such large sections is too great.

The solution to this problem is found in the employment of celloidin as an embedding material. The schedule of dehydration and embedding differs in no way from that usually used. Unfortunately, no way has been found in which to dehydrate with butyl alcohol and embed in celloidin, for celloidin is not soluble in butyl alcohol.

Serial sections of celloidin material are more tedious to obtain than serial sections of paraffin material. Different ways of maintaining the series have been proposed and all are of value. The simplest method and the one which we use is to remove the sections from the knife with a small piece of onion skin paper and place the sections with the paper in a pile. The pile is then wrapped in a larger labelled piece of paper and placed in a jar of 80% alcohol for future staining. Just before staining, each section in series is removed, one corner of the celloidin blotted, and a number stamped upon it. For this purpose, an ordinary date stamp, a used stamp pad, and some mimeograph ink are all that is needed. The 2 rows of numbers in the center of the stamp are left in place and rest is cut off. A stamp pad which has been used until the ink needs renewing is the best. Mimeograph ink is applied to the surface of the pad until enough is added to impregnate the pad. It will be found that numbers applied in this way will remain visible for some time if the sections are NOT LEFT TOO LONG in the clearing fluid. Sections mounted with numbers applied in this way 2 years ago still have numbers showing.

The proposed paraffin technic has been used during the past 3 years on approximately 500 pieces of tissue including rat and dog jaws, human single extracted teeth, and human autopsy material with 1 to 6 teeth. The single teeth were cut for student laboratory examination and from each tooth an average of 100 sections at 7 micron were obtained. Blocks of autopsy material containing, 1, 2, and 3 teeth were cut either bucco-lingually or mesio-distally with great success. Tissues from experimental rats were the most successful and those from dogs the least successful.

Although oral calcified tissue has been the chief object of the study, other calcified tissues of the body may be just as successfully prepared in this manner. Tissues such as rib, vertebra, and long bones sectioned extremely well following paraffin embedding.

SUMMARY

A technic for the preparation of oral calcified tissue for histological examination is presented. This technic differs from other available methods in combining 3 steps which are (1) decalcification by means of a formic acid-sodium citrate reagent, (2) dehydration with n-butyl alcohol, and (3) embedding in paraffin. Excellent sections at 7 microns were obtained following employment of the method on rat, dog, and human jaws. Other calcified tissue has been successfully treated in the same manner. Celloidin embedding is used only when the pieces of human or dog tissue exceed 3 teeth in situ.

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THE EFFECT OF ACUTE STARVATION UPON THE APPOSITION OF ALVEOLAR BONE ASSOCIATED WITH THE EXTRACTION OF FUNCTIONAL ANTAGONISTS[1]

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A detailed description of generalized skeletal osteoporosis resulting from acute starvation in young adult male albino rats was presented in a previous report (1). It was also noted that when destructive changes occurred in alveolar bone as the result of inflammation, the severity of these local changes was accentuated by the existence of a generalized skeletal disturbance, such as that resulting from acute starvation. Recognizing these facts, it was decided to investigate the effect acute starvation would have upon bone formative tendencies which might arise in alveolar bone because of changes in the local environment. To study this problem, it was necessary first to alter the local environment in an effort to stimulate the apposition of alveolar bone; and second, to superimpose the effect of acute starvation upon the bone changes which might result from the altered local environment.

EXPERIMENTAL PROCEDURE

Twenty male adult albino rats were used as experimental animals. The maxillary left first, second, and third molars were extracted from all the animals in order to permit the occlusal movement of the mandibular molars on this side in an effort to stimulate apposition in the underlying alveolar bone. Immediately following this operation, the animals were divided into 2 groups; one group (Extraction Group) was fed a stock diet of Purina Dog Chow Checkers, and the other (Extraction plus Starvation Group) was deprived of all food with the exception of water. Acute starvation in the latter group merely served as one of many possible methods of altering the systemic background. It was selected first, because of its demonstrated effectiveness in the production of generalized skeletal osteoporosis (1), and second, because its severe nature provided a uniform systemic influence. At the end of 8 days all the animals in both groups were sacrificed and the jaws removed, fixed in formalin, decalcified in an equal mixture of formic acid and sodium citrate and embedded in paraffin.

This procedure afforded material for microscopic study in which only the environment had been altered in one group of animals (Extraction Group) while both the local environment and the systemic background were altered in the other (Extraction plus Starvation Group). The findings in the latter group

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would differ from those in the Extraction Group by the degree to which the systemic disturbance would affect the local response of the alveolar bone.

In order to limit the experiments to the specific problem involved and avoid secondary factors which might arise in the course of a prolonged experimental period, the earliest changes in the bone supporting the teeth from which the antagonists had been extracted were considered.

MICROSCOPIC BONE FINDINGS

Extraction Group

Alveolar Bone Beneath the Molars From Which the Antagonists Were Extracted: In all the animals in this group, similar bone changes were observed along the bone margin in the fundus of the alveolar socket (*figs. 1* and *2*), along the interdental septa (*fig. 3*) and beneath the bifurcation of the molar roots (*fig. 4*). In all of these regions elongated trabeculae of non-lamellated bone were found along the alveolar bone margin immediately beneath the periodontal membrane. Occlusal movement of the molars accompanied the formation of these bone trabeculae. Such trabeculae were not observed on the left side of the jaw where there had been no experimental extraction. The matrix of the trabeculae took a basophilic stain and was demarcated from the underlying bone by an irregular basophilic line. The lacunae were large and spherical and each contained an osteocyte with a prominent deeply staining spherical nucleus. At the periphery of the trabeculae was a thin border zone of pale pink granular osteoid matrix which was separated from the periodontal membrane by a discrete beadlike layer of prominent polyhedral osteoblasts varying from 2 to 4 cells in width.

The underlying bone differed from the non-lamellated trabeculae in that it stained more deeply basophilic, lamellae were prominent, the lacunae smaller, flattened and elliptical and the osteocytes correspondingly thin and elongated.

Extraction plus Starvation Group

Alveolar Bone Beneath the Molars From Which the Antagonists Were Extracted: In all these animals elongated trabeculae were observed supporting the mandibular molars from which the opposing tooth had been extracted. Similar trabeculae were not seen on the unoperated side. Occlusal movement of the molars accompanied the formation of the trabeculae. These trabeculae were similar in distribution to those observed in the Extraction Group but presented pronounced qualitative differences. In the Extraction plus Starvation Group the periphery of the bone matrix was markedly granular for a considerable depth into the trabeculae and presented generalized zones of minute fragmentation and dissolution (*figs. 5, 6, 7a* and *7b*). In these peripheral areas the osteocyte lacunae were enlarged with irregularly distorted outlines and in many instances were devoid of osteocytes. Peripheral dissolution of the bone matrix resulted in loss of bone substance and reduction in the width of the newly formed trabeculae. Destruction of the lacunar walls released osteocytes into the surrounding connective tissue.

Fig. 1. Animal 29. Extraction Group. Fundus of alveolar socket, mandibular molar, showing lining of non-lamellated bone separated from underlying bone by clear-cut linear demarcation. H and E × 114 (orig. mag.)

Fig. 2. Detail *fig. 1.* Non-lamellated bone matrix lined by bead-like strand of polyhedral osteoblasts. Note difference between bone structure on each side of dark linear demarcation. H and E × 460 (orig. mag.)

Fig. 3. Animal 28. Extraction Group. Interdental alveolar septum between first and second mandibular molars showing non-lamellated bone formed along mesial aspect and gingival bone margin. H and E × 100 (orig. mag.)

Fig. 4. Animal 28. Extraction Group. Bifurcation of molar roots showing elongated trabeculae of non-lamellated bone beneath the periodontal membrane. H and E × 82 (Orig. mag.)

A bordering layer of osteoblasts separated the bone trabeculae from the surrounding periodontal membrane. The osteoblasts were reduced in number in comparison with the Extraction Group and were entirely absent in many areas. A linear zone of eosinophilic matrix was seen at the border of the trabeculae immediately beneath the osteoblasts. This zone could be demarcated from the

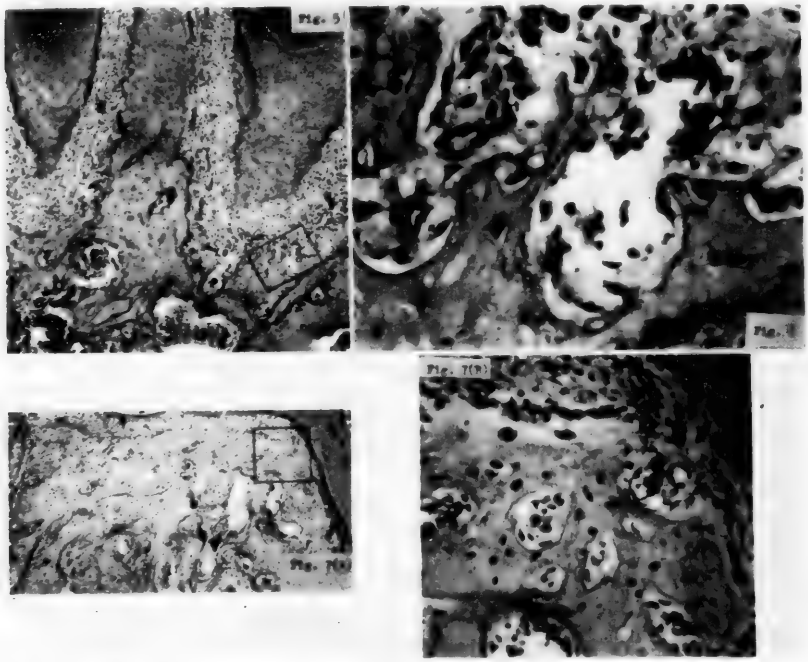

Fig. 5. Animal 25. Extraction Group. Fundus of alveolar socket, mandibular molar, showing lining of non-lamellated bone separated from underlying bone by clear-cut linear demarcation. H and E × 108 (orig. mag.).

Fig. 6. Detail *fig. 5* showing fragmentation and dissolution of bone matrix, irregularly enlarged lacunae, and absence of bead-like layer of osteoblasts. Compare with *fig. 2.* H and E × 720 (orig. mag.).

Fig. 7. Animal 34. Extraction plus starvation Group. A. Bifurcation of molar roots showing non-lamellaetd bone trabeculae beneath periodontal membrane. H and E × 60 (orig. mag.). B. Detail of insert, showing minutely fragmented bone matrix with irregularly enlarged osteocyte lacunae underlying peripheral osteoid border. H and E × 620 (orig. mag.).

underlying matrix of the trabeculae which was fragmented and granular in this group (*fig. 7b*) instead of blending with a homogeneously calcified matrix as was observed in the Extraction Group (*fig. 4*).

DISCUSSION

The findings in both experimental groups represent the response of alveolar bone to an altered local environment. The occlusal movement of the molars

and the apposition of underlying bone were associated phenomena arising from the extraction of the antagonists in the opposing jaw. It is not possible to suggest which of these phenomena is the cause and which the effect. Since by removing the opposing teeth, continued eruption of the mandibular molars was permitted beyond the previously existent occlusal plane, the associated bone apposition may be considered an accentuation of bone changes related to the eruption of teeth under physiological conditions (2).

It is apparent that the attempted accommodation of the alveolar bone to the occlusal movement of the mandibular molars was upset by systemic interference in the Extraction plus Starvation Group. This interference took the form of destruction of bone substance which had already been laid down in an effort to comply with the altered local environment. In addition, osteoblasts which occurred associated with the continued formation of new osteoid matrix were reduced in number.

It should be recognized that the absence of food in the Extraction plus Starvation Group reduced the actual functional stimulation transmitted to the underlying structures during mastication and swallowing. Because the experimental procedure provided for the removal of opposing functional teeth in both groups the added difference resulting from the actual absence of food substance in the Extraction plus Starvation Group would be expected to be minimal.

A significant fact to be derived from the findings is that bone changes which occurred as the result of a local environmental change in one group of animals (Extraction Group) differed from those which occurred when a systemic disturbance was superimposed upon this local change (Extraction plus Starvation Group). The morphological differences between the bone in the 2 groups are tangible indices of the extent to which a systemic disturbance altered a local bone response.

In addition to demonstrating morphological differences in the bone of the 2 groups, the specific osteoporotic tendencies induced by starvation served as a tracer mechanism whereby systemic control over the local formative response of alveolar bone was revealed. The significance of the observations lies, therefore, not in the fact that they revealed differences in a local bone response but that these differences made evident the presence of a systemic regulatory mechanism which could not have been detected in the local formative response of bone in normal animals. The findings did demonstrate the specific manner in which this systemic regulation appears in a severe generalized skeletal disturbance, such as that which results from acute starvation.

CONCLUSIONS

A series of animal experiments was conducted in order to determine whether the deposition of alveolar bone in compliance with local environmental changes is actually a simple local bone response or whether it is in some degree dependent upon systemic regulation. Extraction of functional antagonists was the method employed to alter the local environment. Acute starvation was induced in order to create a generalized skeletal disturbance.

The findings revealed that there is inherent in alveolar bone an accommodation potential which is activated by local changes. When functional antagonists are removed from teeth, this accommodation potential takes the form of bone apposition in an attempt to maintain the anatomical relationship of the teeth and the underlying structures and thereby prevent the roots from losing their support.

Although the apposition of alveolar bone is activated by local influences, it is regulated by systemic tendencies. This systemic regulation is actually capable of changing the microscopic structure of the bone formed to accommodate an alteration in the local environment.

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AN ACQUIRED PIGMENTED PELLICLE OF THE ENAMEL SURFACE

I. Review of Literature[1]

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Among the local factors concerned with the occurrence of dental caries is the presence, on the enamel surface, of organic coverings which may either afford protection to the underlying enamel or be instrumental in initiating decay. These coverings are of 2 fundamentally different types. One is anatomical and is known as "Nasmyth's membrane" or the "enamel cuticle." This is supposed to consist of a primary and secondary enamel cuticle (1), formed during developmental and eruptive stages of the teeth, and may be preserved in part on the enamel and exposed cemental surfaces of the fully erupted dentition. The other type is acquired, and consists of various organic deposits accumulated on the teeth to form adhesive "plaques" or "films" often mistaken for the enamel cuticle.

Technical difficulties encountered in the investigations of both types of coverings account for the divergence of opinion as to their respective structure, nature and significance. It is the purpose of this study to differentiate between the anatomical structure, the enamel cuticle, and acquired plaques or films.

ENAMEL CUTICLE

Berzelius (2) apparently discovered the removal of a membranous structure from teeth immersed in acid. His findings are reported by Retzius (3) who agreed that the floated membrane lined the dentinal side of the enamel. Both failed to recognize its location on the enamel surface.

The first description of the membrane in its true relationship to the enamel surface is credited to Nasmyth (4). He reported that the membrane floated from human and animal teeth by the action of acid was a remnant of the dental capsule (dental sac) which, instead of being deciduous, was functional throughout the life of the tooth; he described it as fibrous externally and reticulated on its inner surface. His concepts of the origin of the membrane, and of its being "a persistent dental capsule" were not accepted by Huxley (5), who thought that the membrane consisted of remnants of the *membrana preformativa*. Nevertheless, Huxley gave Nasmyth recognition for his work and referred to the structure as "Nasmyth's membrane."

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The controversy over the origin of the enamel cuticle is not conclusively settled. Chase (6) reviewed the literature on this question in 1926. The cuticle, or one of its constituents, was described as originating from:

1. the dental sac
   a. persistent capsule (4)
   b. coronal cementum (7 to 10)
2. the enamel organ (1, 5, 6, 11 to 28)
3. stratified squamous epithelium
   a. gingival (6, 14, 29)
   b. gingival crevice (33)
   c. epithelial attachment (1, 18, 19, 30, 31, 32)

The hypothesis that the membrane is derived from the dental sac has definitely been abandoned; the controversy now appears to be centered on what constituents of the enamel organ are involved in cuticle formation, and whether stratified squamous epithelium contributes to it.

Gottlieb (1, 18, 19) and others (30, 31, 32) have expressed the opinion that the enamel cuticle consists of 2 distinct cuticular elements: one, the "primary enamel cuticle," deriving from the ameloblasts, the other, the "secondary enamel cuticle" ("dental cuticle"), originating from the epithelial attachment. Glickman and Bibby (33), assuming that this secondary cuticle is formed from the epithelium of the gingival crevice, recently suggested for it the term, "transposed-crevicular cuticle." Chase (6) believed that the inner pellicle of Nasmyth's membrane originates by fusion of the cuticular borders of the ameloblasts, whereas the outer cellular portion is derived from remnants of the enamel organ and cells from the gingival epithelium. Saunders, Nuckolls and Frisbie (28) concluded from their study of amelogenesis that the enamel cuticle originates from the surface layer of the enamel matrix with which incompletely keratinized cells of the *stratum intermedium* may be incorporated.

There is also a difference of opinion regarding the structure of the enamel cuticle. Nasmyth's first description of an externally fibrous and internally reticulated membrane is more suggestive of a floated "plaque" than of the anatomic cuticle. Most authors agree with Huxley's (5) later description of a thin, structureless, transparent membrane which may retain on its inner surface the impressions of the enamel prisms. Chase (6) contended that these markings on the inner surface are not indentations but remains of detached portions of enamel adherent to the cuticle. Many believe with Paul (13) that the cuticle described by Huxley is only part of Nasmyth's membrane, and that there is superimposed on it one or more layers of epithelial cells. It is generally admitted that the epithelial component of the enamel cuticle is prominent on unerupted teeth, present to a lesser degree on teeth in early stages of eruption, and tends to disappear in later stages. Whether these cellular elements are epithelial remnants of the enamel organ (13, 17, 20, 21, 22) or are cells which have become artificially detached from the epithelial attachment during extraction (19, 30, 32) may depend on the tooth's eruptive status. The latter concept seems to offer a satisfactory explanation for the presence of epithelial

cells on the outer layer of the floated membranes from erupted teeth of young individuals. The opinion of Paul (13) that these cells are remnants of the enamel organ probably is true of unerupted teeth.

The persistence of the enamel cuticle is controversial. Some (6, 16, 22) believed it to be destroyed soon after tooth eruption; others (17, 19, 20, 30, 32) claimed that it is maintained throughout the life of the tooth, at least in protected areas. Most agreed that wear accounts for its partial disappearance. Such disagreement may be traced to the fact that plaques floated from extracted teeth have probably been mistaken for the anatomic membrane. According to Chase (6), the membrane is a vestigial, transitory structure, in the majority of cases destroyed during the early stages of eruption, and probably never found at any considerable time after eruption is completed. Gottlieb (19) pointed out that the secondary cuticle fails to appear in some instances and develops fully in others. Partial or complete calcification of the primary cuticle causes its almost invariable disappearance along with the enamel during decalcification of the specimen. Glickman and Bibby (33) observed a persistent cuticle at the enamel surface adjacent to the cemento-enamel junction in the majority of 64 erupted teeth studied in decalcified sections. It was less frequently seen at the bottom of the occlusal grooves of posterior teeth.

The function and significance of the enamel cuticle is bound to its longevity. Speculations relative to its significance are based mainly upon consideration of its composition. The primary argument in favor of its being keratinous is its histogenesis. The enamel organ epithelium is a specialized surface epithelium; that its cells have preserved their primitive function as keratin-producing cells has been emphasized (28). It is conceivable that the primary cuticle derived from these cells is keratinous. Moreover, there is a definite analogy between the formation of this cuticle and the formation of the organic structure of enamel. The latter, according to Rosebury (34), is a keratin. If the primary cuticle is keratinous, it should exhibit the resistance to acids inherent in keratinous matter, and be expected to serve as a protective covering for the enamel surface.

Gottlieb (19) denied that the primary cuticle is keratinous or exhibits any resistance to acids, since it is usually calcified. His arguments in favor of a relationship between enamel cuticle distribution and susceptibility to dental caries are based on the presence of the secondary or dental cuticle which, by reason of its origin from the stratified squamous epithelium of the epithelial attachment, may be partly or wholly cornified. Thus, the degree of immunity to caries would depend upon the presence of the secondary cuticle and, to a greater extent, upon its degree of keratinization.

In addition to the protective function attributed to the enamel cuticle, it may possibly function as a dialyzing membrane. Pickerill (35) suggested that it separates calcium salts from saliva during post-eruptive hardening of enamel.

ACQUIRED PLAQUES AND FILMS

In the course of the investigations which led him to formulate his theory of the chemico-parasitic nature of dental caries, the attention of Miller (36) was

directed to "decay of the enamel cuticle." In an early delineation of "bacterial plaque" he described this particular condition as a bacterial invasion of the cuticle, bringing about its thickening and eventually its destruction, so that, in the last stages, only a mass of bacteria remains. Following Miller's fundamental contribution to the subject, Williams (37) and Black (38) studied further the conditions which enable bacteria to stay in contact with the enamel surface long enough to initiate acid production. Williams demonstrated, in a series of ground sections, the presence of a thick, felt-like mass of acid- forming micro-organisms covering incipiently decayed enamel. He believed that this mass of "fungi" was instrumental in maintaining contact between bacterial acids and the enamel. Black postulated that "caries of the teeth has its beginning where the conditions are such that micro-organisms causing caries form gelatinous plaques by which they are glued to the surface of the teeth." He described the plaque as a "thin, transparent film that generally escapes observation, and which is revealed only by careful search." He differentiated this plaque from *materia alba*. The formation of gelatinous plaques by "caries fungi" did not occur in every mouth though the organisms may have been growing. Bacterial films occasionally were observed without the occurrence of decay. The formation or failure of formation of the plaque was attributed to an unknown factor in saliva.

Black's concept of the significance of plaques in caries production was not accepted by Miller (39, 40) who stated that bacterial plaques were essential neither to the beginning nor to the production of caries. On the basis of *in vitro* experiments, films apparently served as a protection to the teeth, though by facilitating the precipitation of fine particles of food upon the surface of the teeth, they could have a deleterious influence. In regard to the nature of the films, Miller confirmed his own previous conclusion that they primarily represent a growth of bacteria in or upon the enamel cuticle. He admitted, however, that they can be found wherever the surface is not kept free from mucus and epithelial or food debris.

Lohman (41) then advanced a theory of dental caries in which lesions were supposed to result from the action of mucus upon teeth; mucin, it was thought, was sufficiently acid to dissolve calcified tissues. This theory was not confirmed by Miller (42), who was unable to find any evidence that mucin from saliva dissolved tooth enamel. Lothrop and Gies (43) pointed out that the same mucin content was found in the saliva of both caries-immune and caries-susceptible individuals. Gies (44), however, reporting Ball's findings, showed that calcium was dissolved from $Ca_3(PO_4)_2$ by mucin solutions.

The idea of a mucinous origin of bacterial plaques came from Kirk (45), who believed that plaques were formed through the precipitation of mucin by the action of lactic acid-producing bacteria. He produced synthetic plaques in a culture medium inoculated with "caries fungi" and found that films were precipitated when the medium became acid. On the basis of this evidence it was generally believe that "mucin plaques" paste the fermenting carbohydrates

directly to the enamel surface and protect bacterial acids from the possible neutralizing action of the saliva.

There is little agreement in the literature on the constituents of acquired plaques, particularly on the nature of the organic matter which constitutes their matrix. Blayney, Bradel, Harrison, Hemmens, Kesel, Miller, Muntz, Stephan and Wach (54 to 59) have studied extensively the clinical, bacteriologic, and physico-chemical aspects of bacterial plaques. They demonstrated that the morphology of the bacterial flora of plaques covering actively carious areas is different from that of plaques covering non-carious areas, the most striking difference being the absence of typical forms of lactobacilli in the latter. A study of bacterial plaques from proximal surfaces of premolar teeth, from the time of their eruption into occlusion until a demonstrable carious lesion developed, indicated a definite increase in the incidence of lactobacilli with the onset of caries.

Stein, Hinck and Hoskins (46) made qualitative chemical tests on "Nasmyth's membrane." However, since their material consisted of membranes floated from extracted teeth immersed in acid, their data were probably chiefly concerned with bacterial plaques These workers reported positive Molish, Millon, xanthoproteic and biuret reactions, and the presence of reducing sugar after hydrolysis. They concluded that the plaques were a mixture of proteins, one of which was a glycoprotein.

Chase (6) found films on the teeth of practically all domestic animals, and on 84% of human teeth. They consisted mostly of cocci and filamentous bacteria in a matrix of unstainable or slightly acidophilic material. Bacterial films were produced, not only on teeth immersed in incubated saliva, but on teeth immersed in distilled water. Chase concluded that the matrix of bacterial films is not derived from the saliva, and consequently is not a mucin as thought by Kirk, but "a product of the vital activities of the bacteria." These films could be detached by acids from the surfaces of amalgams and synthetic fillings as well as from that of the teeth. They withstood fairly vigorous brushing for some time, but could be removed by prolonged action, especially if a tooth paste or mild abrasive was used. Their insolubility in water, dilute or strong acids, and dilute and strong alkalies was as great as that of the enamel cuticle.

Dobbs (47) undertook *in vitro* studies of the resistance of human enamel to acid decalcification, and observed that extracted teeth are decalcified first on clean worn surfaces. Cleaning the teeth with pumice and motor-driven brush did not destroy the acid-resistance of unworn surfaces, but tryptic digestion did. He concluded that there are protective membranes on unworn enamel which are organic in nature. These membranes are acquired after eruption, he believed, since unerupted teeth do not exhibit the same acid-resisting property. Dobbs later (48) described these protective films as firmly attached to sound, carious or whitened enamel, and to fillings as well. Their consistency varied from a soft adhesive mass to a dense firm membrane; they exhibited a grey-to-brown pigmentation. The mucin content of the plaques ranged from 27 to

52%. He produced similar plaques *in vitro*, and was of the opinion that mucus collected on the teeth, particularly during sleep, is denatured by bacterial activity and transformed into a firm, resistant plaque. Dobbs further stated that mucin plaques may present a favorable environment for acids to accumulate and decalcify the enamel, and consequently that mucin in the saliva is probably a predisposing factor in dental caries. Diffusion experiments on isolated plaques led him to conclude that weak alkaline salts of saliva do not pass through them in sufficient amount to control acid: hence, that alkaline mouthwashes are useless. On the other hand, soluble carbohydrates in the mouth diffuse through them and are eventually changed to acids.

Bibby (49, 51, 52) and Bibby and Van Huysen (50) studied the local factors concerned with passive defense against dental caries, and found that teeth studied were covered by a film of organic material which apparently provided some sort of barrier to the penetration of acids. This material appeared to be non-keratinous and to contain little mucin. The film, removable from the enamel surface by acid flotation, contained masses of bacteria which were considered to be its principal constituent. It contained a basal layer of pigmented amorphous material, which may have been either a transformed enamel cuticle or something in the nature of a consolidated product of the degeneration of material accumulated on the teeth. Bibby thought that the basal layer may have been formed by processes similar to those giving rise to the hyaline areas found in other parts of the body. The basal layer failed to take any definite tissue stain, showed a light-to-brown pigmentation, and was apparently continuous with fragments of the organic matrix of the enamel.

Plaque material, particularly samples obtained directly from the mouth, have not been adequately investigated chemically. Campaigne and Fosdick (53) reported nitrogen percentages which seem to indicate that the plaque material from the mouth (7.7 to 11.2% N) is not of the same composition as that produced *in vitro* (3.2 and 3.6% N).

Two deposits not directly conforming to the usual concept of bacterial plaque are occasionally found in the mouth. The first was studied by Bibby (61), who named it "pigmented dental plaque." This usually appears as a brown line on the gingival third of the teeth, and may also be present in pits, fissures and depressions on the tooth surface. The brown line is most easily observed on the linguoproximal aspects of the posterior teeth, particularly of the maxilla, and has a tendency to recur after removal. The deposit is similar to salivary calculus, and its presence is usually an index of low susceptibility to caries.

The second nonconforming deposit is the so-called "green stain." This is characterized by a light-to-dark-green discoloration of the gingival third of the upper anterior teeth in children, and is occasionally seen in poorly kept adult mouths. Its occurrence is classically connected with the persistence of shreds of the enamel cuticle, the green pigmentation being attributed to the action of chromogenic bacteria attached to the remnants. This, however, has never been demonstrated satisfactorily. Ayers (62) did not agree with the general belief that cavities usually develop beneath green stains. She was rather of the

opinion that the stain protects the labio-gingival enamel which it covers. On proximal surfaces, where it is sheltered, it may contribute to caries through the action of its own bacteria.

Recently, Manly (60) observed a structureless, recurrent dental deposit which apparently had not been studied before. This film differed from other acquired plaques in being comparatively free from micro-organisms. It was a pellicle-like, brownish deposit found on the teeth of persons who did not use dentifrice abrasives in their oral hygiene. It gave positive protein tests and was highly resistant to chemical reagents, suggesting a keratin composition. This deposit was similar in many ways to the structureless pellicle of the enamel cuticle. Its classification as a plaque in the present review seems to be justified by the fact that it is acquired and recurrent.

SUMMARY

Organic coverings of tooth enamel may be divided into 2 main classes: anatomical structures, and acquired plaques or films. The anatomical, known as Nasmyth's membrane or the enamel cuticle, is formed during developmental and eruptive stages, and remnants sometimes persist throughout the life of the tooth. This membrane is structureless, tough and apparently keratinous in composition. Acquired plaques or films consist of various organic deposits accumulated on erupted teeth. Largely bacterial in composition, their significance in caries production is still a matter of controversy.

The structureless, pigmented pellicle recently described by Manly is comparatively free from micro-organisms. Formed on the teeth of persons who do not use dentifrice abrasives, it tends to recur after removal. It resembles the enamel cuticle in its high resistance to chemical reagents, suggesting a keratin composition.

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AN ACQUIRED PIGMENTED PELLICLE OF THE ENAMEL SURFACE

II. Clinical and Histologic Studies[1]

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The appearance of a peculiar brown deposit on the teeth of individuals who do not use dentifrice abrasives has recently been reported (1). This pigmentation, which apparently had not been studied previously, was related to the use of a liquid dentifrice, the active ingredient of which is an alkyl sulphate (2).

Distribution of Stain. A clinical study of this condition was carried out on a group of eighty subjects, made up almost exclusively of medical students approximately 25 years of age. There were 6 women and 74 men in the group. Each mouth was examined before the experiment for the presence of any previous staining. All subjects were requested to replace their usual dentifrice by a specific liquid dentifrice. Each individual continued using his own toothbrush in the same manner as in the past. Examinations were continued every week over a period of at least 6 weeks. The presence of any discoloration was recorded at each examination. At the end of the experiment the subjects were classified in the 3 following groups: Class 1. Marked staining; discoloration clearly defined, Class 2. Faint staining; discoloration not visible to unpracticed eye, Class 3. No staining.

The distribution of subjects into various groups is shown in Table I.

Two out of 3 who had been using the liquid dentifrice exclusively for 6 weeks presented a faint-to-marked discoloration. The incidence of staining among persons who smoked was greater than among those who did not; however, there is no indication that the habit is more than a predisposing factor in stain production.

The time necessary for the discoloration to appear varied from a few days to 2 weeks. In the greater number of cases, it was evident in 10 days. The intensity of the staining was generally found to increase with time. Where the stain failed to appear in 3 weeks, it did not develop subsequently, and non-staining subjects were still negative when dismissed at the end of the experiment.

Origin of Stain. To determine whether the liquid dentifrice was responsible for the appearance of the stain, 6 subjects with a marked tendency to stain were selected for a special experiment. They were given a thorough prophylaxis and requested to exclude all dentifrice from their daily mouth care. Water alone was to be used as a lubricating medium for the toothbrush. After 1 to 3 weeks

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all 6 showed a pigmentation similar in every respect to that obtained when liquid dentifrice was used (*figs. 1 and 2*). However, the rate of formation appeared to be somewhat slower. This may be explained by a subconscious increase in the thoroughness of brushing to compensate for the lack of a dentifrice, or by the possibility that less lubrication makes brushing more effective.

TABLE I

Classification of 80 individuals on the basis of staining

	CLASS	NO.	MALES	FEMALES	SMOKERS	NON-SMOKERS
1	Marked stain	36	34	2	25	11
2	Faint stain	18	15	3	8	10
3	No stain	26	25	1	8	18

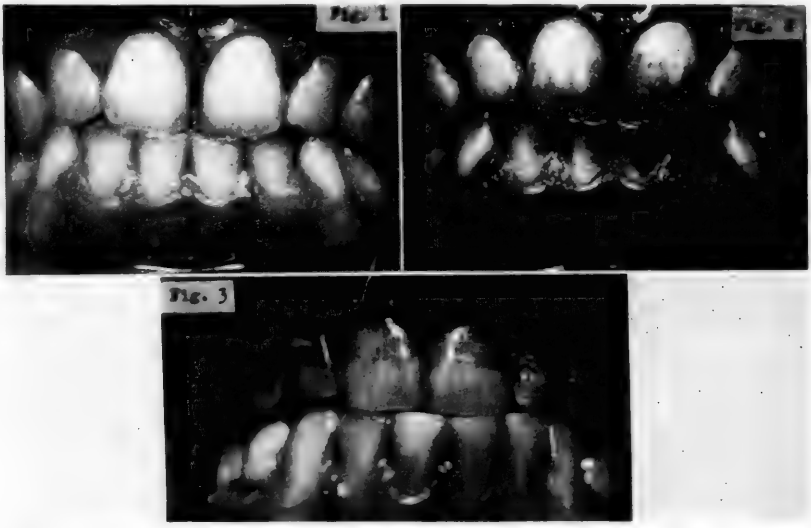

Fig. 1. Mouth condition prior to the experiment. Preliminary prophylaxis was given in this case.

Fig. 2. Same mouth 2 weeks later. With this subject the pigmented pellicle could be obtained at will in 2 weeks' time when either a liquid dentifrice or water only was used with toothbrush.

Fig. 3. Pigmented pellicle involving mostly gingival half of maxillary anterior teeth. Marked inter-proximal deposit.

In spite of the small number of subjects, these results clearly indicated that liquid dentifrice was not causing the pigmentation, but was merely unable to prevent it. Since it was repeatedly observed that brushing the teeth with a mildly abrasive toothpaste or toothpowder would not only prevent but remove the stain, it was concluded that the explanation for its formation was to be found in the absence of abrasive in liquid dentifrice.

CLINICAL FINDINGS

The stain developed into distinct clinical types (substantiated microscopically) depending on the general state of mouth cleanliness. On teeth free from any deposit and debris and kept in that condition throughout the experimental period, the only apparent change was progressive darkening. The teeth did not lose their natural gloss. This fact suggested the presence on the tooth surface of a very thin, translucent, evenly pigmented film, the surface of which was highly lustrous (*fig. 3*). No subjective feeling of uncleanliness resulted. Examination of stained maxillary incisors under the high power binoculars of an ophthalmoscope revealed a thin pigmented pellicle which would be peeled from the enamel with an instrument. This pellicle conformed closely to the irregularities of the underlying enamel surface; perikymata were well outlined by the pigmentation. When the pellicle was observed on teeth in unhygienic mouths it was found to be unevenly deposited, less translucent, rough, often associated with soft deposits.

There were many individual variations in localization of the pellicle. It was most frequently observed on the labial surfaces of maxillary anterior teeth, usually not on the entire surface but on the gingival or incisal third, with a definite line of demarcation between pellicle-free and pellicle-covered areas (*fig. 3*). It was also found on the labial surfaces of mandibular anterior teeth, on the buccal surfaces of premolars and molars (*fig. 5*), and on the lingual surfaces of most teeth. In a few instances the pigmentation had a mottled appearance. As a rule, the enamel surface directly adjacent to the gingiva was free from the deposit. Well-polished fillings and highly glazed porcelain surfaces were always free from it (*fig. 5*).

Discussion. Variations in location cannot be satisfactorily explained. Scratches seen in the pellicle on the incisal and occlusal thirds of the teeth are presumed to be of masticatory origin.

HISTOLOGIC FINDINGS

Method: The pellicle was scraped with a scaler applied gently to the teeth by longitudinal strokes. Each stroke detached a narrow strip of pellicle which was carefully unfolded with teasing needles in a drop of distilled water on a slide. The smear thus obtained was dried in the air. No fixative was necessary since the dried pellicle was found to be sufficiently adherent to the slide to allow any subsequent histologic treatment.

Fresh smears were examined microscopically and carried through general and specific tissue staining procedures.

Staining Reactions. Smears of the pellicle were stained with hematoxylin-eosin, methylene blue, Gram's acid fuchsine, gentian violet, safranine, Mallory's aniline blue, Pianese III b, van Gieson's connective tissue stain, Giemsa, Mayer's mucicarmine, Mayer's muchematein, Hoyer's thionine, Sudan III, Sudan IV (*figs. 4 and 5*).

The pellicle was found to be generally acidophilic. It stained deeply with eosin, acid fuchsine, picric acid, orange G, and other acid dyes. However, it

was also faintly stained with certain basic dyes such as safranine, gentian violet and basic fuchsine. Particular attention was given to so-called specific staining reactions. Staining for mucin, keratin, amyloid substance, fats, and bacteria was made.

1. *Mucin Stains:* Although the specificity of such staining methods is not completely reliable, fairly satisfactory results may be expected. Freshly secreted mucin stains better. A—*Mayer's muchematein stain:* This stain is supposed to give better results with mucin of fibroblastic origin than with mucin of epithelial origin, which may or may not stain. Only mucin should stain blue. The pellicle stained faintly pink, so did desquamated epithelial cells. No blue or bluish color was obtained in any instance. B—*Mayer's mucicarmin stain:* This stain is useful particularly for mucin of epithelial origin. Only mucin stains red. The pellicle was either unstained or stained faintly pink to red. The reaction was always faint, at best doubtful. C—*Hoyer's thionine stain:* Mucin tends to stain more or less intensely with certain basic anilin dyes, particularly with thionine, which gives a typical metachromatic reaction. Mucin should appear light to dark red or purple, or even blue, depending upon the intensity of the stain. The pellicle was mostly unstained. Occasionally it showed a faint bluish stain, but never any typical red, purple or even blue. By contrast, mucus masses present in the scrapings appeared deep blue. The result may be considered as negative. D—*Mallory's aniline blue collagen stain:* As we shall see in detail below, under staining for keratin, this stain should bring about distinct coloring for keratin (orange yellow) and mucin (blue). The pellicle never showed any blue stain, but constantly showed the orange color characteristic of keratinous substances. The results obtained in staining for mucin were negative, or at best doubtful. This by no means indicates that mucin can be ruled out as a possible component of the pellicle. Chemical changes due to aging may prevent typical staining reactions for the substance.

2. *Keratin Stains:* Several dyes have been reported to give uniform and constant staining reactions with keratin; these can be considered as reliable, if not specific. Four composite stains were used, namely: Mallory's aniline blue collagen stain, Pianese III b stain, van Gieson's picric acid and acid fuchsin stains, and Giemsa stain. A—*Mallory's aniline blue stain:* Several references were found, particularly in the dental literature, regarding the staining of various keratinous substances with Mallory's. Orban (6) reproduced a colored drawing of a von Ebner's micro-photograph showing that young enamel epithelium of pig embryo takes an orange color with this stain. Chase (7) pointed out that this stain imparts analogous color to comparable strata of decalcified developing enamel and plantar skin epithelium. Karshan (8) studied more specifically the staining reactions of keratin and concluded that it is stained orange with Mallory's. Previously, Turley (9) had found the same for the corneous layer of skin and the inner hair root sheath. Rosebury (10) confirmed Chase's work and reported that all body surface keratins (nail filings, scurf, skin scrapings, albino hair) take the orange color. He was unable to obtain, however, the same constant results with dental enamel protein and neurokeratin

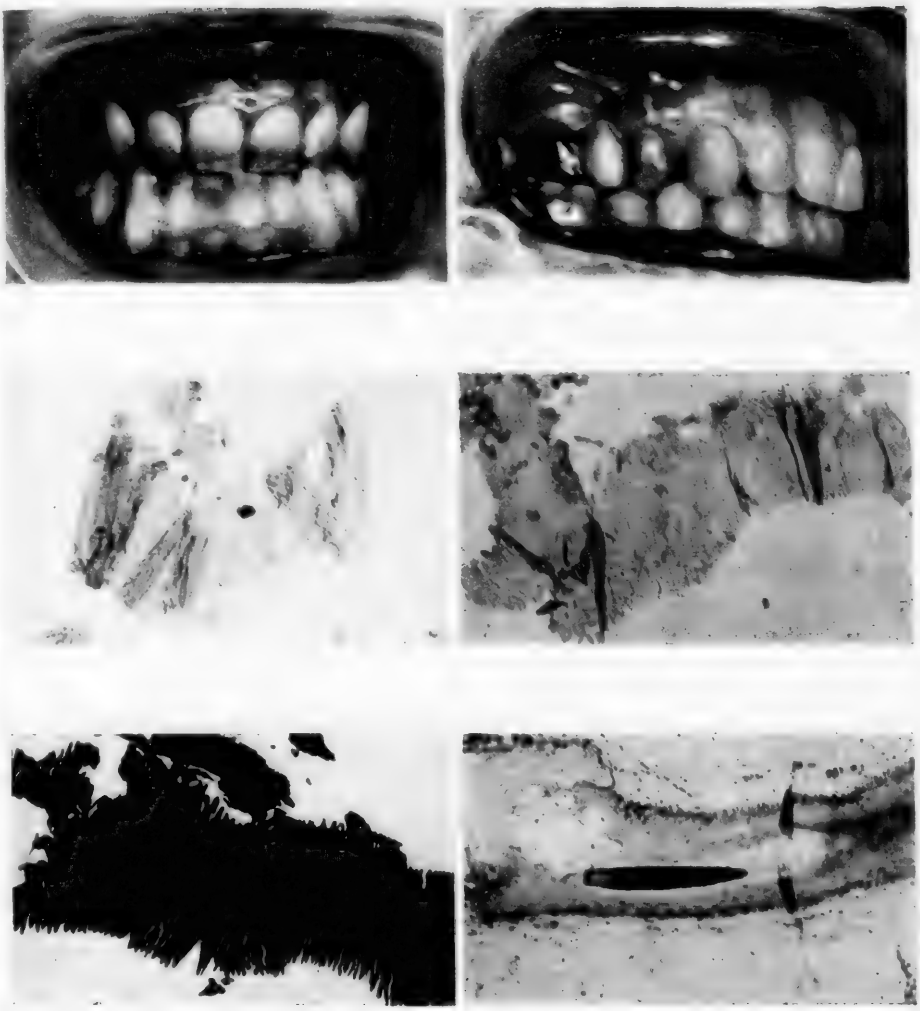

Fig. 4. (Upper left) Pigmented pellicle involving particularly incisal third of maxillary anterior teeth. Marked deposit on mandibular right 1st incisor which is in lingual position.

Fig. 5. (Upper right) Involvement of latero-posterior region. Porcelain facing of maxillary right 2nd premolar does not show any staining, except for its gingival portion which had been ground.

Fig. 6. (Left center) Scraping ×150 (orig. mag.). Mallory's aniline blue.

Fig. 7. (Right center) Scraping ×150 (orig. mag.). Gram's. Pellicle free from bacteria. Compare with *fig. 10.*

Fig. 8. (Lower left) Scraping ×150 (orig. mag.). Giemsa. Compare staining with *fig. 9.* Notice regularly spaced breaks.

Fig. 9. (Lower right) Human skin, hair root ×150 (orig. mag.). Giemsa.

which, according to his work, stained variably from blue to yellow. On the basis of these references one can assume that, at least for all body surface keratins, Mallory's stain gives a uniform and constant orange staining. The pellicle was found to stain uniformly and constantly orange-yellow (*fig. 6*). Skin sections, treated the same way, showed the same reaction for the corneous layer of the epithelium. Nail filings responded similarly. B—*Pianese III b stain:* Rosebury (10) described this stain as giving a uniform and constant green staining to all keratins including neurokeratin and dental enamel keratin. No green staining of either pellicle or cornified layer of keratin was obtained. Both showed staining ranging from rose-blue to lavender, supposedly characteristic of collagen substance. However, the fact that skin keratin likewise did not stain green suggests that the dyes used were unsuitable. C—*Van Gieson's picric acid and acid fuchsin stain:* This stain should give a brilliant red for collagen, a yellow color for cornified epithelium. It was, therefore, considered of interest in this study. The pellicle took constantly and uniformly the yellow stain. So did the cornified layer and the hair in skin sections carried simultaneously. D—*Giemsa stain:* This stain is occasionally used to bring out certain structures in skin epithelium. The pellicle showed a very typical deep blue color (*fig. 8*). An identical color was found exclusively and constantly in the keratinous substance of skin hair root (*fig. 9*). The cornified epithelial layer of the skin sections did not show the blue stain. The above findings indicate a tendency for the pellicle to take up dyes known to be more or less specific for keratin. This point is further substantiated by the similarity of response shown by the pellicle and such keratinous structures as hair, nail, and cornified skin epithelium, when submitted to identical staining methods.

3. *Amyloid Stains:* The possibility of a degenerative substance such as amyloid being a constituent of the pellicle was investigated. Fresh smears were first submitted to iodine (Lugol) which brings out a mahogany-brown reaction with amyloid. This reaction was negative. Topical applications of iodine in the mouth proved to be equally unsuccessful. Smears were stained also according to Mayer's amyloid stain technique with crystal violet. This dye should give a characteristic metachromatic reaction with amyloid (reddish violet). Although the pellicle stained faintly violet, no tendency toward any reddish shade could be determined, and we regard the reaction as negative.

4. *Fat Stains:* Sudan III and Sudan IV failed to stain the pellicle. It should, therefore, be regarded as free from fatty material.

5. *Bacterial Stains:* Bacterial stains, Gram's stain in particular, have been found valuable in this study as a means of differentiating the pellicle as a structure. Tooth surface deposits such as bacterial plaques, masses of desquamated epithelial cells, and mucus clamps are readily identified in scrapings of enamel surfaces stained by Gram's method. A—*Gram Stain:* This stain was used preferentially to methylene blue. Safranine was used as a counterstain. The pellicle, when obtained from clean labial surfaces, was found to be remarkably free from bacteria (*fig. 7*). Scrapings from unclean areas contained fragments of pellicle, filamentous bacteria, and cocci. Most of the cocci were Gram-

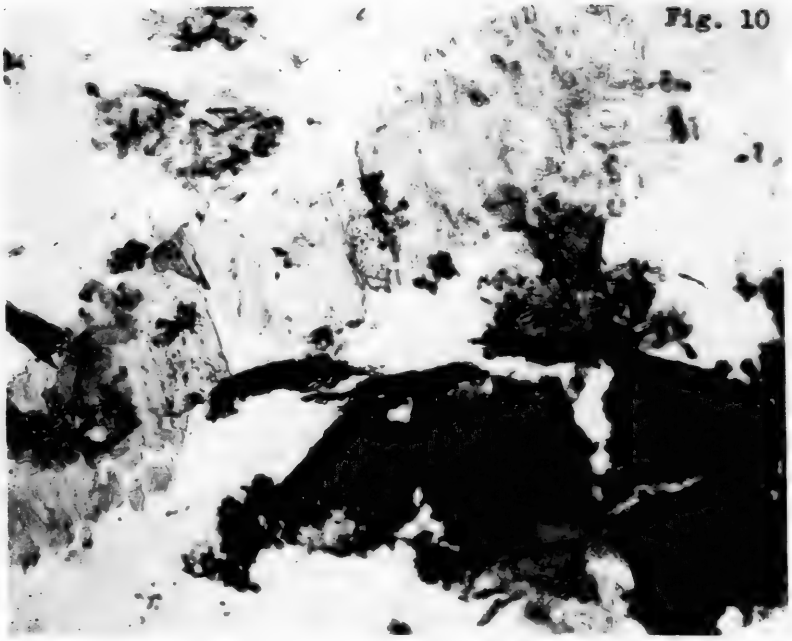

Fig. 10. Smear ×150 (orig. mag.). Gram's stain. Mixed type of deposit. Pellicle associated with masses of Gram positive cocci and bacteria.

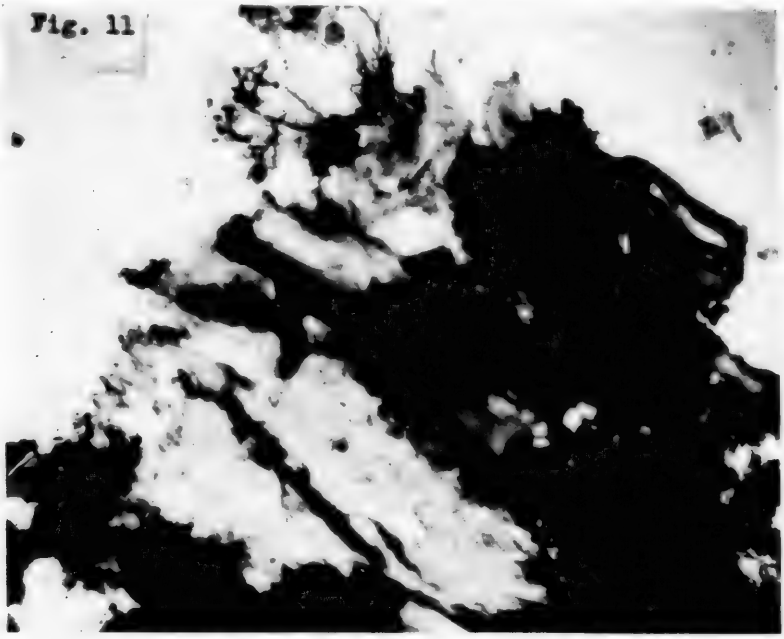

Fig. 11. Scraping ×315 (orig. mag.). Gram's stain. Mixed type of deposit. Pellicle underlying masses of micro-organisms.

positive. The number of organisms seen ranged from a few scattered individuals to compact masses (*figs. 10 and 11*). Desquamated epithelial cells were often identified, either isolated or in groups.

Microscopic Features. Fragments of a thin, translucent, homogeneous pellicle were identified in stained and unstained smears. The pellicle did not exhibit structure, but frequently showed regularly spaced bands of uniform width (*figs. 7, 8*). There is evidence that these parallel breaks in the pellicle correspond to perikymata on the surface of the enamel. Illustrations and diagrams of the microscopic surface relief of enamel presented by Wolf (3) substantiate this finding. Only a few scattered micro-organisms and isolated desquamated cells were observed in addition to the pellicle.

Scrapings from unclean areas where the deposit was thicker and relatively opaque presented a composite picture in which fragments of pellicle were found associated with compact masses of micro-organisms, clumps of mucous material and plaques of desquamated cells (*fig. 10*). In these cases the pellicle appeared to underlie a bacterial film (*fig. 11*).

DISCUSSION

This investigation presents clinical and microscopic evidence that, under certain conditions, the teeth surfaces may become coated with a thin, structureless, translucent, organic pellicle, light brown to black in color. Although this pellicle is similar in many ways to the cuticular layers of teeth, the circumstances of its formation indicate that it belongs in the category of acquired plaques and films. Plaques have classically been described as composed primarily of bacteria, and no reference was found regarding an organized enamel surface deposit devoid of them. However, a membranous structure bearing a distinct resemblance in morphology, pigmentation, staining behavior and acid resistance, was found by Bibby and Van Huysen (11) to constitute the basal layer of acid-resistant bacterial plaques. This observation, based on the study of microtome sections of floated plaques, offers some ground for the suggestion that the pellicle deposition may represent the initial step in the formation of this type of plaque. Secondary deposition of bacteria on the pellicle (*figs. 10 and 11*), and subsequent organization and consolidation could bring about the condition observed by these authors. In plaques found on old teeth, which have resisted decay for many years, the bacterial layer was seen to be separated from the enamel surface by a band of amorphous material. Bibby was of the opinion that both the tooth environment and the enamel organic matrix participated in the formation of these basal layers. The evidence herewith presented indicates the possible mechanism of tooth environment participation.

The information gathered so far in regard to the actual nature of the pellicle material is somewhat contradictory. Its organic nature has been indicated by the preliminary work of Manly (1), who found a positive xanthoproteic reaction. The possibility of fatty or amyloid components was ruled out by staining reactions. On the basis of the marked insolubility of the material in strong hydrolytic solutions, Manly held it to be keratinous. This idea appeared

to be further substantiated by the keratin-like staining reactions obtained in this investigation. These findings should be considered insufficient to warrant such conclusions. Formation of keratin on teeth without specific cellular activity appears impossible from a biological standpoint.

There are good arguments in favor of participation of mucin in the formation of the organic pellicle. Mucin is ever-present in the tooth environment. It has been found by several authors to be a constituent of certain types of mouth plaques, and others have been able to obtain mucin plaques by incubating teeth in saliva. Since mucin could not be demonstrated by specific staining and could not account for the keratin-like staining behavior exhibited by the material, it is suggested that it is not present as such. It is conceivable that a thin film of mucin spreads on tooth surfaces, due to the lack of abrasiveness of the liquid dentifrices, and, being alternately bathed in mouth fluids and partly dried by the passage of air, undergoes changes that may alter its reaction to stains and chemicals. Unless the film is destroyed by periodic brushing with abrasives, layers gradually accumulate to form a pellicle-like structure. The enzymatic activity of mouth bacteria, possibly mediated through the constant contact of the oral mucous membrane, might contribute to the process of alteration of the mucin.

The period of clinical observation covered by the present work has not been long enough to allow any conclusive discussion of the practical significance of the presence of such a pellicle on tooth surfaces. Obviously, its pigmentation makes it esthetically undesirable.

Another interesting angle of this problem appears in the differences in susceptibility shown by subjects of the same age group Apparently some unknown factor in the teeth environment of certain subjects determines the formation of pellicle which cannot be prevented or removed without resorting to the use of an abrasive.

SUMMARY

A thin, structureless organic pellicle was found on teeth surfaces in a large percentage of individuals who used no abrasive in their daily mouth care. A brown-to-black pigmentation appeared simultaneously.

The pellicle could be differentiated from any known enamel surface structure. Some similarity to keratin in staining properties was observed. Staining for mucin, amyloid substance and fat were negative. The mechanism of its formation and its possible significance were discussed.

The author acknowledges the assistance of Mr. M. Orser with the photographs. He expresses his gratitude to Dr. W. R. Bloor and Dr. K. E. Mason who provided the facilities of their respective departments.

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AN ACQUIRED PIGMENTED PELLICLE OF THE ENAMEL SURFACE

III. Chemical Studies[1]

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Previous observations (1) led us to consider the possibility that the pellicle concerned in this investigation was presumably composed of a denatured or otherwise altered mucin. However, from its insolubility in strong hydrolytic solutions, it was held by Manly (7) to be keratinous, an idea further substantiated by the keratin-like staining reactions obtained.

METHOD

The pellicle was scraped from tooth surfaces of selected subjects who rapidly formed abundant material that was relatively pure. The scrapings were collected in distilled water on a microculture slide, where they were separated from visible impurities. The pigmented material was then aspirated into capillary tubes and stored in the icebox. When a sufficient amount of material was believed to be on hand, the content of the tubes was made into a single sample. The yield was necessarily small. The collection of the 2 samples used for analysis required the constant co-operation of an average of 15 suitable subjects over a 3-month period.

A sample of acid floated plaques was also obtained to provide a basis of comparison with the mouth material. Thirty non-carious mandibular incisors were immersed in 5% HCl after thorough scrubbing with a toothbrush and water. The floated plaques were then collected and washed by centrifuging in several changes of distilled water.

RESULTS

Comparative weight and water loss data are given in Table I. Wet sample weights were obtained after removing as much excess water as possible with filter paper. Pellicle sample A and the floated plaque sample were left for 2 hours *in vacuo* at 100°C. over P_2O_5. Pellicle sample B was left 3 hours.

Although comparable H_2O losses were obtained for pellicle and floated plaque, the texture of the latter was macroscopically different, and its whitish appearance contrasted with the dark brown pigmentation of the pellicle samples.

Spectrum Analysis. Qualitative and quantitative spectrum analysis of the pellicle sample A gave the following results:

[1] This investigation was supported by grants from the Proctor and Gamble Company and from the Carnegie Corporation of New York. Received for publication April 2, 1945.

			%
P	40 micrograms		3.2
Ca	10	"	0.79
Mg	10	"	0.79
Pb	5	"	0.4
Sn	Several	"	
Ag, Mn	Few	"	
Na, Cl, Fe, Si	Traces		
K, Sr, Rb, As, Cu, Co, Ni, Zn, Bi, Al	None		

The presence of a comparatively high amount of Pb in the pellicle appeared of particular importance, as this might account for the pigmentation. Qualitative analysis performed later on pellicle sample B and floated plaque sample also revealed the presence of Pb, further confirming this impression. A higher Fe content was expected since a steel scaler was usually used for scraping off the material. The presence of Ag may be explained by the occasional use of a silver scaler.

TABLE I

Comparative weight (mg.) and H_2O loss

	PELLICLE SAMPLE A	PELLICLE SAMPLE B	FLOATED PLAQUE
Net weight wet	3.230	9.464	3.418
Net weight dry	1.265	3.394	1.270
H_2O loss	61%	64%	62.9%

Microanalysis. Pellicle sample B was used for N, C, H and ash microdetermination. The percentage of ash, when burning with $K_2Cr_2O_7$, was given as 24.8. The ash was taken from the C and H determination. An analytical procedure for phosphorus was followed from there. For comparison, N, C and H values were also obtained for the acid-floated plaque. The results are indicated in Table II.

Nitrogen percentages of pellicle and floated plaque were compared with the values found by Campaigne and Fosdick (2) for mouth and synthetic mucin plaques (Table III). Percentage compositions of some mucins and keratins were also charted for comparison (Table IV).

A comparative study of these data indicated that the nitrogen content, as well as the general percentage composition of pellicle material, fell within the range of mucin values. No absolute significance, however, could be given to this finding because of the little margin between the respective values of keratinous and mucinous substances.

Fluorescent microscopy study. Fluorescent microscopy is based on the property, exhibited by certain substances, bodies or tissues, of becoming self-luminous when exposed to the invisible ultra-violet light. Since the quality of the fluorescence, generated by ultra-violet rays, depends upon the chemical constitution of the material under observation, this technique was used in the present investigation, in an attempt to gain qualitative information

TABLE II

*Percentage Composition of Pellicle and Plaque Samples**

	SCRAPED PELLICLE TOTAL SAMPLE	SCRAPED PELLICLE ASH-FREE SAMPLE	ACID FLOATED PLAQUE SAMPLE
Nitrogen	8.90	11.8	7.45
Carbon	38.09,† 37.60‡	50.0	43.30
Hydrogen	5.05, 5.59	7.4	7.27
Oxygen	—	30.8	—
Ash	24.80		—
Phosphorus	4.97§		—

* With the size samples taken the accuracy is −0.5 of 1%; our precision is −0.5.

† Burned with $K_2Cr_2O_7$ to release CO_2 formed with the metals as carbonates. Evidently no carbonates were formed.

‡ Burned without $K_2Cr_2O_7$ and found % ash—ca 24.8, probably as oxides.

§ The ash was taken from the C and H determination and an analytical procedure followed for phosphorus from here. The phosphorus result may be low if any came over during combustion. However, I believe the quantity of the metals held all phosphorus.

TABLE III

Comparative Nitrogen Percentage

SCRAPED PELLICLE	ACID FLOATED PLAQUE	MOUTH MUCIN PLAQUE*	SYNTHETIC MUCIN PLAQUE†
11.8	7.45	11.20 7.75	3.28 3.64

* Ash free basis.

† Campaigne, E. E., 2d, Fosdick, L. S. (2).

TABLE IV

Percentage Composition of Some Mucins and Keratins

SUBSTANCE	ORIGIN	REFERENCE	N	C	H	S	O
Mucin	Submaxillary	Hammarsten 1888 (3)	12.32	48.84	6.80	0.84	31.20
Mucin	Salivary	Lothrop 1912 (4)	10.7				
Mucin	Salivary	Inouye 1930 (5)	13.8				
Mucin	Salivary	Fosdick 1938 (2)	7.76				
Mucin	Salivary	Fosdick 1938 (2)	6.30				
Mucin	Salivary	Fosdick 1938 (2)	3.66				
Keratin	Neuro (brain)	Bogue, R. H. 1922 (6)	13.15	56.99	7.53	1.87	20.46
Keratin	Skin	Bogue, R. H. 1922 (6)	16.64	51.53	7.03	2.18	22.32
Keratin	Hair	Bogue, R. H. 1922 (6)	17.14	50.65	6.36	5.00	20.85
Keratin	Nail	Bogue, R. H. 1922 (6)	17.51	51.00	6.94	2.80	21.75

on the nature of both pellicle and pigment. A new type of lamp provided a source of filtered ultra-violet rays free from all undesirable visible light. An ordinary microscope was used with dark-field condenser. Fresh preparations only were examined, since histologic handling of specimens is likely to destroy

their ability to fluoresce. Contact between the condenser lens and the lower surface of the slide was established with a non-fluorescent immersion oil.

The pellicle showed regular areas of blue fluorescence bordered by zones of red, porphyrin-like luminescence. Nail and skin scrapings examined for comparison exhibited a definite, uniform green color. Desquamated epithelial cells present in pellicle scrapings appeared also to give a greenish fluorescence. This finding seemed to indicate a difference of chemical constitution between the pellicle and the two keratins examined for comparison. The appearance of zones of red fluorescence suggested the possibility of porphyrin in the pigment and led us to investigate this point further.

Investigation for porphyrin. A preliminary experiment was carried out with the Harderian gland of the rat in order to work out a suitable method. It was found that a centrifuged acid-alcoholic extract of the gland presented a marked red fluorescence when exposed to ultra-violet rays. The same method was used for a sample of the pellicle. The extract thus obtained failed to give any red fluorescence. The pigmentation appeared to be unchanged by the procedure. This experiment seemed to indicate that porphyrin was not present in the pellicle.

Investigation for blood pigment. It was felt that decomposition products of blood pigment could possibly account for the pigmentation. A Teichmann test was made on several samples of the pellicle. No Teichmann crystals could be identified. The pigment appeared unchanged by the procedure. This negative result eliminated with reasonable safety the possibility of hematin as the cause of pigmentation.

Bleaching with H_2O_2. Direct applications of 30% hydrogen peroxide on the surface of stained teeth did not bring about any bleaching. The same negative results were obtained by carrying the reaction under the microscope.

Solubility and bleaching with HCl. Increasing concentrations of HCl were used. It was found that the pellicle was highly insoluble. Concentrated HCl did not bring any appreciable change in the pellicle structure. Only by heating did the material go into solution. As for the pigment, it also appeared to be highly insoluble, and a minute amount of black residue was left in the crucible after complete dissolution of the material by heating the acid. This indicated that the pigment was of a very insoluble nature. It also seemed to eliminate the possibility of inorganic metallic salts as a possible cause of pigmentation.

DISCUSSION AND CONCLUSION

Although necessarily limited in scope by the very small amount of available material, the present investigation gave additional information on the nature of the pellicle. It confirmed Manly's preliminary report (7) to the effect that the pellicle consists of a highly insoluble protein. It also seemed to support our previous hypothesis that this protein was a denatured or otherwise altered mucin.

The keratin-like behavior of the pellicle, which had been reported previously (7, 1) and which was also evident in the resistance to concentrated HCl shown

by the pellicle in this investigation, could be accounted for by the chemical changes of mucin rather than by a chemical identity of pellicle and keratin. This opinion, based earlier on theoretical considerations, appears further substantiated by the results of microanalysis and fluoresence microscopy.

The data concerned with the study of the pellicle's brown pigment do not give any definite indication of its nature. Porphyrin and hematin pigments have been eliminated. The possibility of the presence of inorganic salts of Pb and Fe also appears remote, because of the insolubility of the pigmented matter. This property is more suggestive of a melanin-like substance of the nature of that obtained by Deakins (8) from a pigmented carious organix matrix of human dentin.

The author wishes to express his gratitude to Dr. H. C. Hodge, who initiated and guided this investigation; to Dr. R. F. Riley for the microweights; to Dr. L. T. Steadman for the spectroanalysis; to Mr. D. Ketchum, of the Eastman Kodak Co., for the microanalysis; to Dr. E. Lowenstein, of the Bausch and Lomb Optical Co., for the fluorescent microscope.

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INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

Proceedings of the Twenty-Third General Meeting[1]

Stevens Hotel, Chicago, Ill.

May 27, 1945

Compiled by HAMILTON B. G. ROBINSON, *Editor*[2]

Ohio State University, Columbus 10, Ohio

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I. Remarks of Retiring President

H. Trendley Dean, National Institute of Health, Bethesda, Md.

The remarks of the retiring President should be brief, limited largely to a report of the general activities of the Association during the year together with such suggestions that might seem pertinent to the welfare of the Association.

The first nine months of the Association year passed uneventfully. Pursuant to the wishes expressed in a resolution passed by the Council during the 22nd General Meeting, an Advisory Council on Dental Research was appointed from the Association membership. Serving at present on this committee are: Doctors Philip Jay, Thomas J. Hill, J. L. T. Appleton, Wilmer Souder and Harold C. Hodge.

Cancellation of General Meeting: On January 5, 1945 the Director of War Mobilization, acting to curtail non-essential railroad travel, requested that conventions or meetings involving more than fifty out-of-town persons and not specifically connected with the war effort be cancelled. The Council acted promptly and voted that our 23rd General Meeting scheduled to be held at Kansas City, Mo., March 17–18, 1945 be called off.

For the purpose of meeting the constitutional provision of Article VI, e.g., that the Association meet at least once annually, plans were made to hold a *Curtailed War-Time Annual Meeting*. In order that Association business might

[1] This "curtailed war-time meeting," held in collaboration with a special meeting of the Chicago Section, was attended by 39 members from 11 sections.

[2] The General Secretary of the Association, E. H. Hatton, rendered invaluable assistance in compiling these proceedings.

be legally transacted, it was essential that a quorum (30 members with representatives of at least 3 Sections included in this number) be present.

The place where a Curtailed War-Time Annual Meeting might be held was thus limited to one of several cities, e.g., places where the Section membership was sufficiently large to permit calling upon local members in numbers sufficient to make up a quorum when combined with such of the general officers, members of the Council and of the Board of Editors who might be able to attend the meeting. As the roll is called this afternoon it is apparent that more than the number required to meet the constitutional provisions for an annual meeting are present. The loyal members of the Chicago Section and the tireless efforts of the General Secretary have made possible the preservation of the continuity of an annual meeting. This 1945 Curtailed War-Time Annual Meeting therefore convenes today in Chicago for the purpose of:

1. Conducting the annual executive proceedings of the Association,
2. Declaring formally that all addresses and papers listed on the official program be declared read by title, and
3. Installing the new general officers.

Business Administration: The slow steady growth of membership in the Association—as of December 1st, 1944, the total membership was 628—is gradually developing and will continue to develop new administrative problems. Recent changes in tax laws require that non-profit organizations such as ours file an income return with the Collector of Internal Revenue. The suggestion is made that consideration be given to an evaluation of the manner of handling the business affairs of the Association. A relatively complete balance sheet of all Association and Journal financial matters should appear annually in the JOURNAL.

To implement this proposal it is suggested that a standing committee of three members responsible to the Council and known as the Committee on Finance and Business Administration be constituted. In order that this committee be represented of the various activities of the Association, it is further suggested that one member be from the Board of Trustees, one from the Council, and one from the Board of Editors, each member to be picked by the group they represent, e.g., the Trustee representative to be elected by the Board of Trustees, the Council representative by the Council, and the member from the Board of Editor's to be elected by that body.

Each member elected should hold office for a term of three years, except that (1) a member elected to fill a vacancy occurring prior to the expiration of a term for which his predecessor was elected shall be elected for the remainder of such term, and (2) the terms of office of the members first elected will specify whether for a one, two, or three years term of office. When a member, furthermore, has served a full term of three he should not be eligible for reelection until after the lapse of one Association year.

This committee might report annually to the Council on the state of the accounts and records of the Association, including the JOURNAL. In initiating

their work the Council should authorize the committee to engage an individual or firm skilled in business administration and accounting for the purpose of examining into and recommending, if necessary, such labor saving devices as would facilitate the handling of the Association's business and for the design and installation of such specialized accounting system as the business of the Association and the JOURNAL might require.

On or about November 1st of each year the Committee on Finance and Business Administration shall recommend to the Council for their approval a certified public accountant or comparably qualified person or persons who shall audit the accounts of the Association, including the JOURNAL, before March 1st of the following year and who shall report the results of the audit promptly to the Committee. The committee in turn will submit their report to the Council through the Secretary.

If in the reorganization of the business affairs of the Association, it is recommended and approved that an officer, or officers, shall be bonded for the proper care and disposition of Association or JOURNAL funds and/or property the expense of such bonds shall be paid for by the Association.

Certificate of Membership: It is recommended that consideration be given to designing a certificate of membership suitable for framing upon which may be inscribed the member's name, the date of his election to membership and such other data as would seem appropriate.

Committee on Necrology: In order that the necrological items conform closely to a given pattern from year to year, it is suggested that a standing Committee on Necrology be authorized.

General: One would be remiss in both duty and gratitude if they failed to pause for a moment as the Association year passes to an end and express their appreciation to the General Secretary. With little clerical assistance and a volume of work increasing with each succeeding year, he has carried on cheerfully and earnestly. This burden should be lightened by providing such assistance as may be required.

In closing, permit me to again thank the Association for according me the privilege and honor of being elected to serve you during the past year.

II. INDEX OF PARTICIPANTS, AND SEQUENCE NUMERALS OF CORRESPONDING ABSTRACTS

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Smolelis, J. M., 22; Stephan, R. M., 23, 24; Thoma, K. H., 25; Van Huysen, G., 26; Vincent, C. J., 26; Volker, J. F., 27; Wah Leung, S., 16; Wainwright, W. W., 28.

III. Abstracts of Papers Read by Title

1. Radioactive calcium and phosphorus uptake by calcified tissues. *W. D. Armstrong, Ph.D., M.D., School of Dentistry, University of Minnesota, Minneapolis, Minn.* Radioactive calcium and radioactive phosphorus were simultaneously administered by stomach tube to a mature rat. The urine and feces were collected over a 5-day period after which the animal was sacrificed. Of the administered isotopes 64.5% of the phosphorus and 28.3% of the calcium were absorbed. The urine contained 7.9% of the phosphorus and 2.3% of the calcium. The relative uptakes of radiophosphorus to radiocalcium were: Skin and hair—1.03; serum—0.69; femur marrow—1.58; femur epiphysis—0.85; femur diaphysis—0.91; incisor dentin—0.95; incisor enamel—1.00; molar dentin —1.24; molar enamel—2.22.

2. Increment of fluorine content of enamel following topical application of fluorides to teeth. *W. D. Armstrong, Ph.D., M.D., School of Dentistry, University of Minnesota, Minneapolis, Minn. and J. W. Knutson, D.D.S., D.P.H., Division of States Relations, United States Public Health Service, Bethesda, Md.* Teeth extracted from the mouths of children who had received 2 to 6 topical applications of 2.0% fluoride applied to half the teeth of the mouth were examined for fluorine content of the enamel. There was no evidence that fluorine content of the entire enamel was increased by the topical applications of fluoride but the possibility that an increase occurred in the fluorine content of the superficial layer of the enamel is not excluded.

3. Histologic study of agramulocytotic lesions of the teeth and their surrounding structure. *William H. Bauer, M.D., D.D.S., St. Louis University, School of Dentistry, St. Louis, Mo.* The jaws of 3 young patients who received bismuth-neoarsphenamine antisyphilitic treatment and died of agranulocytosis with septic infarcts in the lungs, were studied in histologic sections. One case showed extensive gangrenous lesions of the oral mucosa and particularly of the gingiva and the tonsils. The 2 other cases, however, revealed only slight marginal ulceration with profusely bleeding and swollen gingivae. Though the occurrence of these pronounced hemorrhages would fit better into the picture of an acute leukemia, the blood and the marrow findings ascertained the diagnosis of agranulocytosis. The more or less aplastic bone marrow of the 3 cases contained lymphocytes and plasma cells, and an occasional reticulo-endothelial hyperplasia, mainly in the 2 hemorrhagic instances, was noted. The interesting histologic findings offered a satisfactory explanation of the question often discussed: Why is it that the marginal and interdental gingivae become involved first or shortly after the lesions appear on the tonsils? It is not, as it is generally thought, a mechanical trauma, so common on the gingiva, that initiates the action of the anaerobic microorganisms. It is in the gingival crevices where these microorganisms lodge that the gangrenous process starts.

From here the gangrene proceeds further into the gingiva, the periodontal membrane and oral mucosa and provokes a necrosis of the alveolar process.

4. NON-REVERSIBLE CHANGES DUE TO OVERDOSAGE OF VITAMIN D IN ORAL AND OTHER STRUCTURES IN THE DOG. *H. Becks, Agnes Fay Morgan, Daniel A. Collins, and Ruth M. Freytag, University of California, San Francisco, Cal.* Severe changes in osseous and dental tissues have been observed to follow chronic overdoses of vitamin D in dogs. The object of the following experiment was to determine whether or not these changes are reversible. Two dogs were fed a purified diet plus intermittent daily doses of vitamin D (10,000/Kg./day) for 5½ months. A total dose of approximately 1,500,000 I.U. was administered during this period. Each dog was allowed a recovery period of 5 months and was then sacrificed, roentgenographed and prepared for histologic examination. The clinical symptoms of cachexia, lassitude, anorexia, polyuria, polydypsia, hyperglycemia, diarrhea, etc., were manifested by these dogs as the result of the chronic hypervitaminosis D. The severe changes in the jaws and teeth included generalized vertical and horizontal atrophy of periodontal bone structure, multiple pathologic calcifications in the pulp chambers, amorphous calcified deposits on the surface of roots and bone trabeculae, severe marginal inflammation, osteosclerosis and atypical cementum formation. No indications of removal of these pathologic calcifications could be detected anywhere in the tissues during the recovery period. Since these histologic changes produced by chronic hypervitaminosis D are identical with those previously reported, it can be concluded that they are not reversible. The warnings by previous investigators regarding the danger of administering chronic excessive doses of vitamin D must be repeated on the basis of these studies until the maximum tolerance dose is established.

5. STUDIES ON ENAMEL SOLUBILITY. *Basil G. Bibby, B.D.S., Ph.D., D.M.D., Michael G. Buonocore, B.S., M.S., and Morris Diener, B.S., D.M.D., Tufts Dental College, Boston, Mass.* The possibility that a variety of anions and cations might influence enamel solubility in the same manner as fluorine and silver has been investigated. To test enamel solubility a gravimetric method using powdered enamel and a variety of acid buffers was employed. A variety of ions such as oxylates, lead, zinc and copper are effective in reducing enamel solubility under the conditions of this test. The effect of lead is much greater than that of fluorine. (Published in *J. D. Res.* **24**: 103, 1945.)

6. TRANSITORY PHASE OF MALOCCLUSION. *Carl Breitner, M.D., D.D.S., New York, N. Y.* Animal experiments and clinical observation have led to the realization that many dento-facial anomalies are, in their early stages, of a transitory nature. If untreated, some of these anomalies will improve spontaneously, while others will develop into permanent deformities. If a case improves spontaneously, we may safely assume that the original cause of the anomaly was a passing one, and, once dissipated, no longer prevented normal development. In most cases of the other variety, that is, those which show no tendency to spontaneous improvement, either the original causative factor may be a permanent one or it may have disappeared, but have left in its trail a me-

chanical arrangement fixating the existing anomaly or preventing normal development. We must, therefore, allow the tendency toward spontaneous improvement of malocclusions to exert itself through the following methods: (1) elimination of a prevailing cause and (2) elimination of remaining mechanical interference. The tendency to self-improvement, without further mechanical treatment, has been demonstrated with most promising results in cases, especially those of Angle's Class II.

7. INHIBITION OF EXPERIMENTAL DENTAL CARIES IN SYRIAN HAMSTERS BY FLUORINE AND IODOACETIC ACID. *Peter P. Dale, D.M.D., and Paul H. Keyes, D.D.S., School of Medicine and Dentistry, University of Rochester, Rochester, N. Y.* Litter mate hamsters averaging 35 days of age were distributed into 4 groups of 10 females and 12 males, each and placed on the Hoppert-Webber-Caniff finely ground corn meal diet ad libitum for 125 days. About 63% of the corn meal passed through a 60-mesh screen, the remainder through a 40-mesh screen. Group I animals served as control and received tap water; Group II received 50 p.p.m. F as NaF in distilled water; Group III, 50 p.p.m. F as NaF in distilled water plus 200 p.p.m. HIac in the food; Group IV, tap water and 200 p.p.m. HIac in the food. All animals received weekly supplements of fresh apples and carrots. Hamsters that received fluorine and/or iodoacetic acid supplements thrived better than control animals. On completion of the experimental period, the hamsters were sacrificed, jaws removed, fixed in 10% formaldehyde solution, dried, stripped of soft tissues, and the molar teeth carefully examined under a binocular (X20) microscope. Lesions were recorded by 2 independent examiners and evaluated by the method of Keyes (*J.D. Res.* **23:** 439, 1944). Caries occurred bilaterally and was most pronounced in maxillary 2nd and 3rd molars. Males seemed more susceptible than females. Average caries scores per animal for the various groups were: I-IV, females, 35.4, 1.4, 0.4, 3.7; males, 49.4, 3.2, 0.4, 9.8. Inhibition of caries activity by fluorine and iodoacetic acid (II, III, IV) was evident. Degree of fluorosis observed in incisors of female animals was correlated with fluoride content. Dry weight whole-tooth analyses in p.p.m. F were: I-IV, 37.4, 983, 1003, 81.5.

8. INITIAL OXIDATION-REDUCTION POTENTIALS OF SALIVA. *L. L. Eisenbrandt, Ph. D., University of Kansas City, School of Dentistry, Kansas City, Mo.* The initial oxidation-reduction potentials (E_h) of unstimulated saliva of 7 subjects tested repeatedly disclosed that this physiological property is a function of time (monthly and seasonal). A total of 1555 electrometric determinations was made bi-hourly from 9 A.M. to 5 P.M. for 1 week in each of 12 consecutive months. The mean E_h of the saliva was +301 mv. and the mean pH was 6.64. Although the pH showed marked individual characteristics, the initial oxidation-reduction potentials of saliva did not vary to any extent. Also, the pH of saliva changed greatly during the day, but the oxidation-reduction potentials did not. Both E_h and pH showed significant variations in the saliva of the group from month to month. The E_h was below the mean during the fall (+248 mv. in November), winter and spring, but was above the mean during the summer, reaching the high E_h of +394 mv. in September, i.e., the warmest period of the

year. The pH of the same saliva was high in the fall (6.72 in October), low at mid-winter (6.56 in January), high in the early spring (6.68 in March) and low again in the early summer (6.53 in May). Seasonal variations in the incidence of caries and Vincent's infection, as well as other diseases, have been reported. A relationship between these pathological manifestations and oxidation-reduction potentials may eventually be established because of seasonal cycles. Also, on the same basis there is a relationship between E_h and the activity of the thyroid gland. High environmental temperature and humidity increase the effects of thyroxin, and likewise E_h is apparently influenced in the same manner. (To be published in the *J. D. Res.*)

9. PHOSPHATE DESORPTION FROM POWDERED BONE AS SHOWN BY THE RADIOACTIVE ISOTOPE. *Marlene Falkenheim and Harold C. Hodge, School of Medicine and Dentistry, The University of Rochester, Rochester, N. Y.* Phosphate is rapidly adsorbed on powdered bone from solutions of Na_2HPO_4 as shown by the radioactive phosphorus isotope. The most rapid phase of this process appears to be over in 30 minutes to 1 hour. If this phosphate adsorption is an exchange, one would expect a rapid removal of marked phosphate from bone when the bone samples are placed in contact with a solution of the same concentration of phosphate, but without radioactive phosphorus present. To determine the rate at which phosphate is desorbed from powdered bone, several 50 mg. bone samples were first exposed at 40° for 1 hour to a solution of $2 \times 10\text{-}3$ M Na_2HPO_4. Then after 2 distilled water washings these samples were returned to a non-radioactive solution of the same concentration at 40° C. for various time intervals. The amount of phosphate retained by the powdered bone and the amount desorbed by the phosphate solution were determined. It appears that the most rapid desorption (50%) took place in the first 15 minutes.

10. CARIES OF THE DENTIN. *H. E. Frisbie, D.D.S., University of California, College of Dentistry, San Francisco, Cal.* Based upon preliminary studies of developmental structure of dentin (*J. D. Res.* **22:** 221, 1943) problem of carious progress into dentin has been followed in recently extracted human teeth. Upon reaching dentin, carious process proceeds laterally along dentino-enamel junction and then retrogressively into overlying enamel, and at same time extends into dentin, but in beginning at slower rate. Some frontal loss of tissue results which is accompanied by deeper penetration by way of tubules. This initial tubular penetration and continued ingress of microorganisms is along calcifying matrix bordering tubules. With penetration of the organisms this matrix exhibits an acidophilic staining reaction. In more extensive superficial positions organisms are found within dentinal fibril and lateral branches. Newly infected lateral branches likewise take acid stain and it may be assumed that fibril in these parts as well as its branches has been injured through enzymatic action following ingress of microorganisms. As process continues acidophilic reaction in border matrix increases in intensity, in this way producing appearance ordinarily described as widening of tubule. Tinctorial change in every way corresponds to that described in caries of the enamel, (*J. A. Coll. Dent.*, **11:** 243, 1944). This change is believed indicative of proteolysis of infected dentin matrix which

releases inorganic salts from their organic bond. With involvement of many lateral tubules and accompanying extension of the proteolytic process coalescence occurs forming localized irregularly shaped areas of disintegration of dentinal structure. Shrinkage at dentino-enamel junction has never been observed in fresh specimens. However, as result of carious process spreading laterally along junction and involving overlying enamel matrix, there is area of proteolysed material in which microorganisms are seen in profusion. Unless extreme care is used to prevent desiccation, artifact having appearance of shrinkage area, is rapidly produced. Immediately beneath what is apparently hard and unbroken enamel surface, areas of brown discolored dentin are found in which late proteolysis is observed and only occasional scattered organisms are present. Peripherally in this zone localized colonies are seen where marginal proteolysis is found and in advanced areas tubules may be invaded to variable extents.

11. PERIODONTAL STRUCTURES IN ALBINO RATS WITH ALLOXAN DIABETES. *Irving Glickman and Anna Morse, Tufts Dental College, Boston, Mass.* This is an initial report of the microscopic findings in periodontal structures of 12 of a series of 30 albino rats in which diabetes was created by 1 or 2 subcutaneous injections of Alloxan in a dose of 200 mg./kg. Blood sugar determinations, made by the Falin-Malmos method upon blood taken from the tail, revealed a hyperglycemia (393–671 mg. %) in each animal. No pathological microscopic changes were seen in the periodontal tissues of animals sacrificed 2 days following injection with Alloxan. In animals sacrificed 42 to 70 days following injection, the gingival and alveolar bone findings were consistent throughout the jaws of each animal but presented individual differences. The gingival picture varied from a slight hyperemia without pocket formation to marked gingival ulceration, extensive pocket formation and the presence of abscesses in the bifurcation region. Bone formation normally seen in relation to the periodontal membrane and endosteal margins was generally reduced. Loss of periodontal bone associated with osteoporosis throughout the jaw occurred in the absence of marked gingival change. Pronounced osteoclasias occurred in relation to gingival inflammation and pocket formation.

12. DENTIN CARIES. *B. Gottlieb, Baylor University, College of Dentistry, Dallas, Tex., and E. Applebaum, Columbia University, College of Dentistry, New York, N. Y.* Caries consists of invasion of 2 groups of microorganisms along the organic routes of the tooth, a proteolytic group and an acidogenic group. The end result of the carious process is the formation of a necrotic cavity, which can be accomplished only by the proteolytic group. The acid component produces definite effects, which can be identified in the enamel as well as in the dentin. In the meager soil of the enamel no microorganisms can develop properly. At the dentino-enamel junction they reach better living conditions and we find their products spreading here, undermining the enamel. The spreading of the yellow pigmentation can be seen only in the ground section. The acid produced makes the dentin shrink away from the enamel, tissue fluid is aspirated and coagulates under thick enamel. Shrinkage under thin enamel results in unsupported enamel edges. The result of acid action shows up in the x-ray picture.

The shrinkage of the dentin here may cause the formation of a split, between the dentino-enamel junction and the dentin. Sometimes a layer of dentin remains in connection with the enamel, separating it from the split. In other cases the split develops in the deeper parts of the dentin, tearing the dentinal tubuli open. Tissue fluid is aspirated from the tubuli here too, and a secondary focus for developing microorganisms is formed. From here they invade the opened dentinal tubuli. Such splits, caused by shrinkage, look like a section through a biconvex lens. The space is filled with the homogeneous tissue fluid and the dentinal tubuli are straight on either side. If, however, an area of liquefaction is formed by proteolytic activity, we find a more rounded cavity, filled with granular necrotic material. The dentinal tubuli are usually curved outwards, indicating an increased pressure in the necrotic area and a softening of the surrounding dentin by acid. Such areas of liquefaction are caused by necrotizing of dentin matrix between neighboring tubuli, along which the microorganisms spread.

13. Relationship of vitamin E to changes in dental and periodontal tissues of the rat incisor. *Humberto Granados, Dr. Odontol., Karl E. Mason, Ph.D., and Henrik Dam, Sc.D., School of Medicine and Dentistry, University of Rochester, Rochester, N. Y.* Maxillary incisors of rats reared on a standard purified E-deficient diet containing 18% lard and 2% cod liver oil for 60 to 78 days after weaning were completely depigmented, but mandibular incisors retained normal yellow pigmentation. Replacing lard with cod liver oil had the same effect on the dental pigment, but substitution with hog liver fat caused only partial depigmentation of upper incisors. Rats on the standard diet 159 to 326 days exhibited the same macroscopic depigmentation, including mandibular incisors, but when given E supplements for an additional 150 days, showed a restoration of normal pigment that appeared at the gingival margin a few weeks after instituting therapy. Recent experiments (Granados, H., and Dam, H., *Science*, **101**:250, 1945) demonstrate that depigmentation of incisors does not occur if fat is entirely excluded from the E-deficient diet from weaning, and suggest an important relationship between fat or fat metabolism and incisor pigmentation that may explain contradictory findings of previous investigators (Davis, Moore, and Irving). Histological sections of jaws of rats fed E-deficient diets mentioned above revealed an increase, proportional to the duration of deficiency, of macrophages containing large number of fuchsinophil globules in enamel organ, periodontal membrane, gingival crest, bone marrow, and adjacent muscle. In staining reactions, distribution, and difficulty of removal after prolonged E therapy, this pigment is the same as that noted by Mason in the musculature and other organs of E-deficient rats, considered to reflect a specific metabolic disturbance. Incisor depigmentation and increased pigmentation of soft periodontal tissues are considered unrelated except for a common relationship to vitamin E functions.

14. Treatment of pulpless teeth with concentrated sulfonamide solutions. *Louis I. Grossman, D.D.S., Dr. Med. Dent., Dental School, University of Pennsylvania, Philadelphia, Pa.* The root canals of 152 teeth were

treated with a concentrated sulfonamide solution after the canals were mechanically prepared and irrigated. The sulfonamide solutions used were (a) 15% sulfanilamide in ethylene glycol; (b) 15% sulfadiazine is ethylene glycol. Ethylone glycol alone was used as a control solution. The "blindfold" method of testing was employed, i.e., those using the sulfonamide solutions or the control solution were not aware of their identity. For diagnosis, the sulfonamide-treated group of 152 cases was compared with another group of 152 cases which had been treated with azochloramid, beechwood creosote, cresatin, and camphorated monochlorphenol. The latter medicaments were used in rotation during the course of treatment. The number of treatments required to yield 2 successive negative cultures was used as a measure of effectiveness of the root canal medicament in each case. The concentrated sulfonamide solutions were found to be almost, but not quite, as effective as other root canal medicaments used for comparison in this study.

15. PRODUCTION AND INHIBITION OF DENTAL CARIES IN THE SYRIAN HAMSTER. II. *Paul H. Keyes, D.D.S., School of Medicine and Dentistry, University of Rochester, Rochester, N. Y.* Shortly after weaning, 100 litter mate hamsters were distributed into 5 groups of 8 male and 12 female animals. Group I hamsters received a basic diet of whole corn; 4 males and 6 females, a bi-weekly supplement of powdered milk and alfalfa. Group II received the Hoppert-Webber-Canniff diet made with semifine corn meal. Group III received the H.W.C. formula (2/3) and confectioners sugar (1/3). Group IV were maintained on the H.W.C. ration, and the molar teeth of each animal received weekly 2-minute topical applications of an NaF solution containing 1:1000 F. Group V received the H.W.C.-sugar ration and similar topical applications of a 50% urea solution. All animals were provided with food and distilled water ad libitum, and weekly supplements of greens. The experimental period was 110 days. Hamsters on whole corn (I) grew poorly and developed few carious lesions; milk and alfalfa supplements improved growth but had no effect on caries activity. Animals on the H.W.C. ration (II) developed a greater amount of caries but grew more rapidly. Sugar (III) resulted in a pronounced increase in caries activity. Topically applied fluoride (IV) apparently reduced caries activity, but urea treatment (V) was not beneficial. The average numbers of teeth affected per animal for the various groups are: for females, I-V, 0.2, 1.0, 8.3, 0.8, 11.8; for males, 1.1, 4.6, 8.6, 1.4, 11.6. Corresponding caries scores: females, 0.1, 0.3, 51.1, 5.8, 111.7; males, 1.8, 19.3, 64.1, 3.2, 172.5.

16. NATURALLY OCCURRING STAINS ON THE TEETH OF CHILDREN. *S. Wah Leung, D.D.S., School of Medicine and Dentistry, University of Rochester, Rochester, N. Y.* A survey of 355 children from several orphanages and reform schools in a middlewestern state disclosed that 80% had deposits of stain of various types on the surfaces of their teeth. The incidence appeared to be slightly higher among boys than among girls. Classified by color, the deposits were green, yellow, orange, brown, and black. Green stain was most prevalent and occurred in 58% of the children studied. Following in the order of incidence were brown, yellow, orange and black deposits. These stains were found singly in most

instances, but multiple occurrences were observed in about ⅓ of the subjects. There appears to be a relationship between the quantity and type of stain, caries activity, and general oral condition.

17. PREVENTION OF DENTAL CARIES BY BRUSHING THE TEETH WITH POWDERED FLUORAPATITE. *J. F. McClendon and Aris Carpousis, Hahnemann Medical College, Philadelphia, Pa.* The teeth of 120 enlisted men of the U. S. Army were examined for dental caries at the beginning and end of a 1-year period during which they were taking medical training. Of these, 40 were given powdered fluorapatite to brush their teeth and 80 were used as controls. Some of the 40 brushed their teeth daily for 1 year with the fluorapatite which contained nearly 4% fluorine. Others neglected its use after a longer or shorter period. The 40 using the fluorapatite developed an average of 0.5 new cavities per man per year whereas the controls developed 1.5 cavities per man per year. Brushing rats' teeth with fluorapatite as reported by McClendon (*J. D. Res* **21:** 305, 1942) has been continued for 3 years. Dental caries are thereby greatly delayed in appearance but not reduced to zero. Rats with teeth brushed daily with fluorapatite from 60 to 100 days from weaning averaged 0.16 carious teeth per rat, whereas litter-mate controls averaged 3 carious teeth per rat. Rats with teeth brushed 100 to 150 days averaged 0.6 carious teeth per rat, whereas litter-mate controls averaged 4 carious teeth per rat.

18. THE EFFECTS OF A SINGLE MASSIVE DOSE OF VITAMIN D_2 ON ORAL AND OTHER TISSUES OF YOUNG DOGS. *Agnes Fay Morgan, H. Becks, Daniel A. Collins and Helen E. Axelrod, College of Dentistry, University of California, San Francisco, Cal.* In recent years "vitamin D shock therapy" was introduced for the prevention of rickets in infants (administration of single large doses of 1,500.000 I,U. of vitamin D). This technique has been employed to treat chronic arthritis, allergies, psoriasis and other diseases. To determine the effect of such acute overdosage of vitamin D on dental and periodontal structures, as well as other organs of the body, 5 purebred Cocker Spaniels were given 1 massive dose of 450,000 I.U. of irradiated ergosterol, each, when 23 and 34 days of age. All manifested the clinical symptoms of acute hypervitaminosis D and were sacrificed at 71, 117, 270 and 272 days of age. Roentgenographically the character and quality of the jaw bone structure were altered and frequent root resorptions were observed. Before eruption the developing permanent teeth showed severe pathologic pulp chamber calcification. Histologically all developing permanent teeth of the youngest dogs showed pathologic calcifications, some nearly filling the entire pulp chambers. The staining properties of the outer layers of the developing enamel indicated a heavier mineral content. Pathologic calcification was observed on the surface of the bone trabeculae and the outer surfaces of the roots. Perivascular calcified amorphous material was frequently seen in the periodontal membrane. Pulp stones were observed occasionally in the deciduous teeth. Similar conditions persisted in those dogs that lived for longer periods after the acute hypervitaminosis D. The roots had developed but pathologic calcifications in the pulp chamber remained. In connection with severe distortion of many permanent teeth communications between the under-

lying tissue and the pulp chamber in the areas of bifurcation were seen and frequently irregular enamel and dentin formation was present. Severe pathologic calcifications were found in the lungs and kidneys. From this study it is evident that the hypercalcemic state immediately following 1 massive overdose of vitamin D produces pathologic calcifications of hard and soft tissues of the jaws and also of some of the internal organs. These acute changes are identical with, if not more severe than, those reported by Becks as the result of chronic vitamin D overdosage.

19. THE ERUPTION OF THE FIRST MOLAR OF THE RAT AND THE SHEDDING OF THE INTERCUSPAL TISSUE. *James Nuckolls, University of California, College of Dentistry, San Francisco, Cal.* Changes in the intercuspal area similar, except in orientation, to those in the cuspal region result in the development of a corium and the formation of an organized epithelial plug with a connective tissue core. Closely following the appearance of the blood vessels along the outer walls of the follicle, new vessels invade the intercuspal stellate reticulum, breaking up the outer enamel epithelium which remains as isolated groups of cells or rests. The invading vessels lay down along with them a connective tissue, similar in appearance to the dental follicle, within the condensed stellate reticulum which almost replaces the epithelial cells with the exception of the single row of the stratum intermedium. Within the intercuspal area the cells of the stratum intermedium, adjacent to the reducing ameloblasts, show a transition from their characteristic flattened form to that of young epithelium and exhibit great mitotic activity. After several layers of cells have developed through this proliferative activity, an organization takes place to form a basal layer and basement membrane. The old cells and those nearest to the formed enamel undergo a keratinization away from the central connective tissue core which now functions as the corium. The cells of the basal layer continue to proliferate in a manner, similar to that occurring in the epithelium overlying the intercuspal region, sending out strands of epithelial cells into the connective tissue core. With the eruption of the tooth and the almost complete keratinization of the intercuspal tissue the blood supply is cut off and the tissue is shed without hemorrhage in the form of a plug keratinized on all surfaces.

20. THE EFFECT OF ACID BEVERAGES CONTAINING FLUORIDES UPON THE TEETH OF RATS AND PUPPIES. *J. S. Restarski, D.D.S., M.D.S., R. A. Gortner, Jr., M.S., Ph.D., and C. M. McCay, Ph.D., Naval Medical Research Institute, Bethesda, Md.** The immersion of an extracted human tooth in one widely consumed "cola" beverage grossly decalcified the enamel surface within 2 days. White rats allowed to drink 20 cc. daily of this beverage exhibited marked destruction of the enamel on the lingual surface of the molars within 1 week. The extent to which the various molars of individual rats were attacked differed from one animal to another. Experiments have indicated that the phosphoric acid contained in this beverage was the principal agent responsible for destruction of the enamel. The beverage studied had a pH of 2.6 and contained 0.055%

*The opinions and views set forth in this abstract are those of the writers and are not to be considered as reflecting the policies of the Navy Department.

H_3PO_4 as well as about 10% sucrose. Solutions of acid plus saccharin were much less destructive to rat molars than those of acid plus sucrose of the same sweetness, viscosity and pH, indicating that sucrose increases the action of phosphoric acid on enamel. Fluorides in concentrations of 1, 6 and 20 p.p.m. F, when included in acid-sucrose beverages, usually decreased the effect of the acid upon the teeth of rats. In some instances, however, fluoride proved ineffective. Puppies allowed to drink 500 cc. daily of an acid beverage 3 weeks before the shedding of their deciduous incisors showed gross etching of the enamel. The same destructive effect of phosphoric acid-sucrose was also observed on the permanent teeth. When the solution contained fluoride, this effect was decreased. Sucrose solutions alone had no effect upon the teeth of rats or puppies. (Published in *J.A.D.A.* 32: 668, 1945).

21. ALTERATIONS OF THE BLOOD PICTURE AND THEIR SIGNIFICANCE IN TREATMENT OF ROOT CANAL WITH INFECTED CONTENTS BY IODINE-IONTOPHORESIS. *M. Salpeter, M.D., Tel-Aviv, Palestine.* In the course of treatment of infected root canal by iodine-iontophoresis (I.I.P.) examinations of the blood picture have been carried out by the author and the inference drawn that the I.I.P. gives rise to immunobiologic processes resulting in the cure of the complete focus. The alterations of the blood picture are similar to that found by Hoff with injection of bacterial proteins with a particular prominence of the "phase of the lymphocyte cure" according to Schilling, i.e., the increase of the lymphocytes and a decrease of the neutrophile cells. The author believes that the I.I.P. is in its effect similar to vaccination with auto-vaccine, as the hyperemia following the I.I.P. brings protein-substances of bacteria, killed or at least weakened by iodine, from the focus into the circulation. This opinion is supported by the onset of large swelling and pains at the periapical area of the tooth being treated. These phenomena are considered by Roessle as a hyperergic inflammation, the way which the sensitized body responds to prevent penetration of the specific toxin brought from the focus into the circulation by the iodine-iontophoresis. In this case the course of the alterations of the blood picture is different from that mentioned above. The characteristic increase of lymphocytes and decrease of the neutrophile leucocytes do not appear until these "blockade symptoms" had subsided and the specific substances from the focus reach the circulation without any obstacles.

22. THE EFFECTS OF TIME AND TEMPERATURE ON THE GROWTH OF LACTOBACILLUS ACIDOPHILUS IN SALIVA. *J. Murphy Smolelis, A.B., Indiana University School of Dentistry, Indianapolis, Ind.* Saliva was collected from 25 students. One c.c.m. from each sample was mixed with 9 c.c. of dextrose broth and $\frac{1}{10}$ of the mixture inoculated on tomato-juice agar according to Hadley's method. A second aliquot was incubated for 24 hours before mixing with broth. The third was held 96 hours at room temperature before mixing and plating. The fourth aliquot was held for 48 hours at 8 degrees C. At the end of a 5-day incubation period the cultures held for 24 hours at 37 degrees C. showed slightly more than twice the number of *L. acidophilus* organisms per c.c. and those samples that were held 96 hours at room temperature showed an average increase of 27 times

the number of organisms found in aliquot A. The fourth aliquot held at 8 degrees showed a decrease in number of organisms per c.c. when compared to the initial culture. The quantitative variation of organisms under the 4 conditions to which the samples A, B, C, and D were subjected was not a constant one in all cases.

23. AN ANTIMONY-SILVER ALLOY ELECTRODE FOR DIRECT pH MEASUREMENTS IN CARIOUS LESIONS. *Robert M. Stephan, M.S., D.D.S., Walter G. Zoller Memorial Dental Clinic, University of Chicago, Chicago, Ill.* Antimony electrodes have proved useful for determining pH directly in the mouth, but due to the brittleness of the metal, this electrode cannot be made small enough in diameter to reach relatively inaccessible carious areas. In search of a smaller electrode, a series of antimony-silver alloys were made and tested. Alloys with a silver content as high as 70% functioned as antimony electrodes. Apparently any antimony in excess of the formula Ag_3Sb is not chemically combined with silver. These alloys were less brittle than antimony itself, although still not strong enough to furnish the ideal electrode. An alloy of 60% Sb and 40% Ag was made into a small pointed electrode which permitted direct pH readings in many carious areas not accessible to the regular antimony electrode.

24. THE pH OF CARIOUS LESIONS. *Robert M. Stephan, M.S., D.D.S., Walter G. Zoller Memorial Dental Clinic, University of Chicago, Chicago, Ill.* This report is concerned with those lesions which have very small openings in the enamel and extensive undermining caries in the dentin. The lesions were diagnosed from bite-wing x-ray films. The tooth was isolated with a rubber dam in order to exclude saliva, and access was gained to the lesion by operative means. The pH of carious dentin was determined in situ with an SbAg alloy electrode. Then some carious dentin was removed from the tooth, placed in a drop of triple distilled water, and the pH immediately determined in vitro with a micro glass electrode. Of 22 lesions thus tested, the pH average was 4.77 in situ, 4.82 in vitro; the pH range was 4.4 to 5.3 both in situ and in vitro. The SbAg and glass electrode readings were in good agreement with a mean deviation of .08 pH. These pH tests were made from 1 to 8 hours after the ingestion of food. They, therefore, indicate that the acidity of relatively inaccessible carious lesions is maintained for a longer time than the acidity of more accessible lesions and plaques. A significant observation was that after the lesions were opened, the acidity of the carious dentin decreased, slowly in situ, more rapidly in vitro.

25. TREATMENT OF FRACTURES AND FRACTURE DISLOCATIONS OF THE MANDIBULAR CONDYLE, WITH DESCRIPTION OF A METHOD FOR OPEN REDUCTION AND INTERNAL WIRING OF THESE FRACTURES, AND ANOTHER FOR SKELETAL FIXATION OF DISLOCATIONS. *Kurt H. Thoma, D.M.D., Massachusetts General Hospital and the Harvard School of Dental Medicine, Boston, Mass.* Thirty-two condylar fractures are reported, 5 bilateral and 22 unilateral. In 16 cases, other mandibular fractures were present. Of these 32 fractures, 3 were incomplete or showed no displacement. In 11 there was overriding with displacement of the condylar fragment; 22 were fracture dislocations of various types, and 3 were old fractures with deformity, such as pseudoarthrosis and ankylosis. The treat-

ment of these fractures was discussed. The various methods in vogue have been described, and it was pointed out that careful checking of the end results did not show that these methods gave consistently good results, as contended by most of the writers on this subject. Contrarily, both major functional disturbances, as well as minor complaints, such as pain, limited motion, and malocclusion, are quite commonly the result of conservative treatment. It was pointed out that in children spontaneous readjustment of the mandibular joint, epiphyseal growth of the condyle, and positioning of the teeth will in time greatly aid in the correction of minor defects following treatment, but that in adults better results can be obtained by operative procedures. A method for open reduction with internal wiring fixation has been described and advocated for cases in which there is wide separation of the fragments favoring non-union, or marked overriding and displacement of the condyle, which would produce shortening of the ramus and open bite. Another method has been described for the reduction of fracture dislocations with immobilization of the condyle by skeletal fixation. The proper handling of concurrent fractures in the horizontal rami has been discussed, as well as methods for immobilization of the jaw until the condylar fractures are united, and functional treatment which should follow to reestablish normal use of the joint before the fractures in the horizontal rami have healed. To this end methods of fixation for such fractures that will allow free motion of the jaw have been discussed. Fourteen case reports in which the advocated methods have been used have been included.

26. RAT CARIES. *Grant Van Huysen, D.D.S., C. J. Vincent, B.S., College of Dentistry, Indiana University, Indianapolis, Ind.* Albino rats were fed for 60 days on a diet containing ground corn which passed through a 20 and stayed on a 40-mesh sieve. The teeth of the sacrificed rats were cut in a sagittal plane by grinding from the distal through to the mesial surfaces with a #52 S.S.W. abrasive point. This operation was carried out under observation with a binocular dissecting microscope (12X magnification) so that it was possible to observe, while grinding, the occlusal fissures, caries or fracture of these fissure walls and the tooth pulp chambers. Caries was observed in 76 of the 141 fissures examined. Only 13 teeth showed cusp fracture. Practically all of the fracture type caries occurred in the lower jaw in the central fissure of the first molar tooth. In the technique used where grinding is accomplished under observation with the microscope one can see that the pulp chamber periphery at these points comes close to the base of the fissure. It was repeatedly observed that caries at this point soon penetrated to the pulp causing enlargement of the cavity by fracture of the weakened tissues. It was concluded from this study that this diet will not cause fracture unless the cusp is first undermined by caries.

27. DISAPPEARANCE FROM THE MOUTH OF INGESTED GLUCOSE. *J. F. Volker and D. M. Pinkerton, Tufts Dental College, Boston, Mass.* The method of Folin and Malmos has been utilized to study the presence of reducing substance in resting saliva. The average value for salivary reducing substances (calculated as glucose) for a group of 30 individuals was approximately 20 mg. %. The disappearance of ingested glucose fed in the form of chewing gum, hard candy

and chewy candy was determined in a group of 12 people. The rate of disappearance varied considerably from person to person but in general was most rapid after gum chewing and least rapid in chewy candy. The maximum concentration of salivary sugar is reached within 5 minutes and the return to normal is usually complete after 30 minutes. Concentrations as great as 4,000 mg. % have been recorded for certain types of candy.

28. CHANGES IN THE ORAL MUCOSA ACCOMPANYING ACUTE PANTOTHENIC ACID DEFICIENCY IN YOUNG RATS. *W. W. Wainwright, and M. M. Nelson, College of Dentistry, University of California, San Francisco, Cal.* Severe changes in the oral mucosa, especially necrosis of the epithelium, was found in rats fed pantothenic acid deficient diets, confirming previous observations on dogs by Becks, Wainwright and Morgan. The diet of the rats in this experiment was more specific for pantothenic acid deficiency than the previous filtrate fraction deficient diet since it included p-aminobenzoic acid, choline, and inositol of the filtrate fraction of the B vitamin group. The other known members of the filtrate fraction, biotin and folic acid, were not included. The rats were placed on the deficient diets at birth and the deficiency was so acute that many of the animals died before 30 days of age. All animals were severely retarded in growth but did not show other deficiency symptoms. In the animals that survived a longer period of time, the characteristic symptoms of the deficiency gradually appeared, usually after 45 days of age. Necrosis of oral epithelium was seen in animals which survived approximately 60 to 100 days or more. As in the earlier observations on dogs, the gingival epithelium was destroyed with a singular lack of inflammatory response even though the necrosis in some instances had reached the subepithelial connective tissue (published in *Am. J. Orthod. and Oral Surg.*).

Abstracts presented by the Materials Group

29. EFFECT OF TIME AND TEMPERATURE ON THE POLYMERIZATION OF ACRYLIC RESIN MONOMER-POLYMER MIXTURES. *Ida M. Harman, Vernon-Benshoff Co., Pittsburgh, Pa.* The paper is a report on the effect of variations in curing technique on the physical properties of a methyl methacrylate denture base. The investigation included the following: variations in both time and temperature of cure; the strength of the resin was compared after curing at a low temperature for periods from 2½ to 72 hours, and after a boiling cure which varied both as to rate of heating and time of boiling. Transverse tests were made on specimens cut from samples 3 m.m. thick. In addition to A.D.A. specification tests, hardness, linear shrinkage, and dimensional stability over 6 months were compared for samples cured by several commonly used techniques. Variations in the size and shape of specimens cured in a water bath of the same ambient temperature were shown to affect the strength of the resin. Pyrometric studies during the cures demonstrated the relationship between the strength and the temperature reached inside the samples during polymerization. Although this

study was carried out with denture resins, the information is equally applicable to the operative materials due to their similarity in composition and behavior. The paper was illustrated with graphs and slides.

30. REDETERMINATION OF THE CASTING SHRINKAGE OF GOLD—A PROGRESS REPORT. *G. M. Hollenbeck, D.D.S., and E. W. Skinner, Ph.D., Northwestern University Dental School, Chicago, Ill.* The accepted value for the casting shrinkage of gold (1–25%) is admittedly in error by + 0.1%. A water-cooled mold has been designed by means of which it is hoped that the accuracy of determination will be increased.

31. SELF-HARDENING LINING MATERIALS. *Carlos Pomes and E. W. Skinner, Northwestern University Dental School, Chicago, Ill.* Six self-hardening denture lining materials, purchased on the open market, have been studied in the testing laboratory and clinic. Their composition is a mixture of a plastic and a solvent, such as ethyl or butyl acetate, or chloroform. Tests for stiffness, loss of weight in air and in water, and warpage showed: (1) the solvent never completely volatilized from the material; (2) the solvent penetrated the polymethyl methacrylate denture base and caused it to soften and warp; (3) the materials did not harden homogeneously, but rather shrank, producing cracks and blebs. It was concluded that the fundamental basis for compounding these materials is wrong, and that they have no place in dentistry in their present state of development.

32. THE PHYSICAL PROPERTIES OF FURNACE MELTED VS. BLOW-TORCH MELTED DENTAL GOLD CASTINGS. *K. W. Ray, General Refineries, Minneapolis, Minn.* Dental appliances and tensile specimens were cast by melting gold in a crucible in a gas-fired pot furnace, and pouring the gold when melted into the crucible of a centrifugal casting machine for casting. The physical properties of the castings were compared to those of the same gold melted by the regular dental blow-torch and cast centrifugally. Also, various pouring temperatures of the furnish melted gold were used, and the properties of the castings determined. The results indicate that ordinary dental methods of melting and casting golds do not always produce castings of maximum quality.

33. FURTHER RESEARCH STATUS OF DIRECT ACRYLIC FILLINGS. *Fred A. Slack, Jr., D.D.S., Philadelphia, Pa.* Direct acrylic fillings have had 2 serious drawbacks. Long hardening time and polymerization contraction have been responsible for most failures. Failures have averaged at least 50%. The graph of specimens made with simple polymer, monomer and accelerators shows contraction occurring approximately 3 days after the pellet was prepared. New pellets corresponding in bulk to fillings were prepared using initial expanding-type monomer. Hardening time was decreased by incorporating organic salts to disperse the accelerator. These pellets show initial expansion curves which remain permanent. These pellets were later tested for hardness and compared to standard Plexiglass specimens. Methods of preparing pellets, measuring and taking their hardness readings are briefly discussed. This same procedure is now being used to place fillings. No clinical results are as yet available.

IV. Necrology

ROBERT K. BROWN (1893–1944)

Dr. Brown was elected to membership in this Association at the Memphis General Meeting in 1931. He was an occasional attendant at the annual meetings especially in the earlier years of his membership and not infrequently participated in the programs on such occasions. He was particularly interested in the application of psychology to the development of aptitude tests and their relationship to the study and practice of dentistry.

He secured his preliminary education at a Pennsylvania normal school and received his D.D.S. degree from the University of Michigan in 1919 as well as a M.S degree in psychology in 1928. Dr. Brown was a valued member of the faculty of his alma mater from 1922 to 1933, attaining the rank of professor. He was engaged in private practice in Cleveland (1919–1922) and in Detroit from 1933 to the date of his death. He was an associate editor of *Dental Survey*.

The subject of this sketch was born at Sharpsburg, Penna., Sept. 22, 1893 and died in April, 1944, and is survived by his widow, one son, Capt. Robert B. Brown, in service, and a daughter.

STANLEY W. CLARK (1887–1944)

Membership in this Association was conferred on Dr. Clark at the Fourteenth General Meeting in 1936. He attended but one general meeting and was listed once as a program participant. He was not actively interested in the activities of the Chicago Section to which he belonged.

He took his dental training at Northwestern University, receiving his D.D.S. degree in 1916 and M.D.S. in 1934. He promptly became a member of the dental school faculty and in time was raised to a professorship of Materia Medica and Therapeutics. He volunteered early for service in World War I and served with distinction in France with the Expeditionary Forces in the Dental Corps. Military affairs claimed his interest from the time of his discharge till his death, he was very active in the organizations of the reserve officers and reached the rank of colonel. He was highly respected by his faculty colleagues and students for his scientific knowledge and sound judgement.

Born in Chicago in 1887, he died in April, 1944, and is survived by his widow and two brothers.

FREDERICK L. STANTON (1874–1945)

Elected to membership at the 1934 Chicago General Meeting, Dr. Stanton participated only in the activities of the New York Section of the Association.

He studied under Dr. W. G. Stewart as preceptor and later matriculated in the New York College of Dentistry (1891). In 1905 he enrolled in the Angle School of Orthodontia at St. Louis and was one of the organizers of the Alumni Society of the Angle School of Orthodontists. From this time on his professional interests and activities were in the field of orthodontia.

V. Executive Proceedings

The 1945 curtailed-war-time meeting of the Association was called to order at 2:15 P.M., May 27 in room 416, Stevens Hotel, Chicago, Ill. by the temporary chairman, George B. Denton, President of the Chicago Section, who after a brief greeting and welcome to the officers and visiting members of the Association transferred the meeting to H. Trendley Dean, President. After verification of the quorum (attendance of 39 members from 11 sections) President Dean declared the meeting open for the conduct of the 1945 executive activities of the Association and called for the reports of the Council, officers and committees.

On motion of the Secretary-treasurer the minutes of the 1944 general meeting were approved without correction as printed in the June, 1944 issue of the Journal of Dental Research.

The reports of the ad interim activities of the Council, the Secretary-treasurer, the Editor and Business Manager of the Journal of Dental Research and of the Treasurer of the Committee on Wm. J. Gies Endowment Fund for the Journal of Dental Research were received, approved and ordered printed in the proceedings. (See pages 209–214).

The report of the tellers (Jasper and Hall) who canvassed the ballots for the 1945–46 active officers was received and the tellers discharged. The electees were verified for induction into office.

The petition of members Crawford, Hooper, Hall, H. B. G. Robinson and Peyton requesting that part (b) of paragraph (C) of section (1) of article (VII) be amended was received and its submission to the Association for a vote was ordered. The draft of the revision follows:

> Each group may have an advisory representative in the Council and in the Board of Editors, each to be selected by the group. If any group shall have ten or more members who do not belong to sections or other groups, its representatives in the Council and the Board of Editors, may, on approval of the Council, be entitled to the same voting privileges as the councillors and editors representing sections.

The necrology report was submitted and the members and their guests stood briefly in a tribute to our late members, Robert K. Brown, Stanley W. Clark and Frederick L. Stanton.

The Council [Attendance—Dean, Armstrong, Chase, Hall (representing Indianapolis Section), H. B. G. Robinson, Hatton, McCauley and Hodge (advisory)] reported that it had inspected the credentials of the persons listed elsewhere (page 215) who had been nominated for membership and had approved them for submission to the Association at the current executive session. It had advised and supported the Editor in his efforts to secure prompter publication of the Journal of Dental Research, even to the extent of changing printers, if that were found necessary. It had approved the report of the Committee on Dental Research and recommended that the incoming President reappoint the committee as now constituted (except for the vacancy caused by the expiration of the one year appointment), and furthermore suggested that the

Council determine by a mail vote whether or not this committee should be made a standing committee of the Association or Council (as provided in the By-laws, Section F). It requested the usual authorization from the Association to act for the Association between this and the next general meeting, especially with respect to the selection of the time and place for the next general meeting, the election of honorary officers and suspension of dues payments by members not resident in North America. And it had examined and approved the budgets requested by the Secretary-treasurer and the Business Manager of the JOURNAL OF DENTAL RESEARCH.

By a unanimous vote the Secretary-treasurer was directed to cast a written ballot for the election of the persons duly nominated and approved by the Council to membership in the Association (total of 17 as published in the proceedings, page 215). Ballot cast.

The budget of the Secretary-treasurer and of the Business Manager of the JOURNAL OF DENTAL RESEARCH for the 1945 calendar were approved formally and tentatively for the 1946 calendar year. (For details see reports as printed herewith, Secretary-treasurer authorized to expend not in excess of $400.00 per calendar year, and the Business Manager up to $8,000 per calendar year.)

Moved and carried unanimously that the annual dues for the 1945 and 1946 calendar years be set at $5.00 per member per calendar year, $1.00 of which is appropriated for the support of the office of the Secretary-treasurer and $4.00 for the expenses of the publication of the JOURNAL OF DENTAL RESEARCH.

Activities of the Council were approved, and on motion the Council was authorized to act for the Association between this and the next meeting in generral and specifically as requested in the Council report.

President Dean then called on Past President Frederick B. Noyes to install the active 1945–1946 officers of the Association all of whom were present to accept their elections. The remarks of both the out-going and incoming presidents were short and limited by the exigencies of the occasion and preceded official adjournment and transfer of the meeting to the temporary chairman, Dr. Denton who for the Chicago Section introduced in turn three program items by George M. Hollenbeck, Balint Orban and Edward H. Hatton.

EDWARD H. HATTON, *Secretary-treasurer.*

Council Activities. In the interim between this curtailed meeting of the Association and the Twenty-second General Meeting at Chicago the Council voted: (a) to hold the Twenty-third General Meeting at Kansas City, Mo., on March 17 and 18, 1945; (b) to approve the establishment of a Tennessee Section; (c) to approve the request of the Secretary-treasurer for a $400.00 budget for the 1944–1945 year; (d) to authorize the Secretary-treasurer to collect dues directly from the members but only for the current year; (e) unanimously to approve the action of the Association members in a legal executive session at the recent (Twenty-second) General Meeting authorizing two changes in the By-laws (see page 209 of the Proceedings and details at end of this paragraph); (f) to remit the annual dues of all members not residing in the United

States of America; (g) to reconsider and cancel all plans to hold the Twenty-third General Meeting in Kansas City on March 17 and 18, 1945; (h) to authorize a committee of five, the President, President-elect, Vice-president, Secretary-treasurer and Editor to make plans for a curtailed executive meeting for 1945; (i) to approve the selection of Chicago for such a meeting place; (j) to validate the votes for general officers (except for members of the Publication Committee) provided the meeting date is later than the 27th of April; (k) to approve extension of the terms of office of the members of the Publication Committee until their successors have been installed; and (1) to declare all ballots cast for the proposed constitutional amendment null and void because of failure to issue official notice within the six months period after its submission. In addition the Council was notified of the formation of the Committee or Council on Dental Research in accordance with directive of the Association at the Twenty-second General Meeting; its members as appointed by President Dean are: Philip Jay (Chairman), T. J. Hill (Secretary), J. L. T. Appleton, Wilmer Souder, and Harold C. Hodge. President Dean recommended that the appointments be made for terms of 5, 4, 3, 2, and 1 years, respectively.

By-law Amendments. (a) Item 1, Part A of Section B, with reference to the payment of dues, is amended to read as follows:

1. Method of collection. (A) Dues of all members of the Association shall be payable directly to the Secretary-treasurer or to his appointed representative. Dues shall become due as of January first of each calendar year.

(b) Section B, Item 3, Part D with reference to exemptions is amended to read as follows:

(D) Rights of membership shall not be affected by any remission of dues, except that members whose dues have been remitted shall receive the JOURNAL OF DENTAL RESEARCH only if the subscription price charged to members has been paid.

A. SECRETARY-TREASURER'S REPORT

Representing this Association the Secretary-treasurer attended three meetings of the executive committee of the American Association of Dental Schools in the interest of correlation of the annual meetings of both associations. He made two trips to Indianapolis to confer with the Business Manager of the Journal of Dental Research, both of these in addition to the routine activities of the office.

As of the current year and effective not later than December 31st, 1945 the following resignations have been submitted and are effective as of the date indicated on the resignation: J. A. Detlefsen, S. M. Gordon, R. H. Ivy, R. W. Leigh, H. A. Miller, G. J. Reed, Adolph Schultz, Benjamin Tishler and Harry E. Kelsey.

As the result of death the names of R. K. Brown, S. W. Clark and F. L. Stanton have been removed from the membership list.

The following members are delinquent for more than one year for dues: E. P. Brady, H. C. Brown, A. J. Bush, Kermit Christensen, Martha Jones, George Stein, Edward Taylor, R. F. Vines and J. L. Zemsky.

The Halifax Section has been carried on the membership and section lists although their resignation was submitted a number of years ago. As the result of some correspondence with one member, it is suggested that one more effort be made to retain as members such as still have an interest in the Association. The resignations of the others should become effective now.

The membership of the Association as of March 15th, 1945, includes 518 names in the North American Division and 103 in other parts of the world, total 621.

Respectfully submitted,
EDWARD H. HATTON, *Secretary-treasurer.*

Financial Report—Secretary-treasurer

Receipts

	1944	*1945 (estimated)*
From general dues	$324.60	$330.00
From gifts (endowment)	128.72	234.93
From interest	40.12	40.00
Total	$493.44	$604.93

Expenditures

Stationery and printing	$140.29	$75.00
Postage and mailing	67.85	70.00
Meetings and travel	66.45	70.00
Clerical and letter service	65.07	150.00
Total	338.66	365.00

On Hand as of May 15, 1945

In General Fund	576.12
In Dental Art and Dental Science Fund	675.57
Unremitted Collections for JOURNAL OF DENTAL RESEARCH	682.00
Unremitted Collections for Chicago Section	33.00
Total (Savings Account #13548 State Bank and Trust Co., Evanston, Ill.)	$1966.69
In Wm. J. Gies Endowment Fund	$1372.23
In JOURNAL OF DENTAL RESEARCH Funds, 2 bonds maturity value of	$50.00

Respectively submitted,
EDWARD H. HATTON, *Secretary-treasurer.*

B. REPORT OF PUBLICATION COMMITTEE OF THE JOURNAL OF DENTAL RESEARCH

Report of Editor for Volume 23, 1944

The year 1944 has continued as a difficult period because our printer has been overburdened with war contracts.

The record given below is for 1942, 1943, and 1944:

	1942	*1943*	*1944*
No. of scientific papers published	63	57	46
No. of abstracts published	127	84	58
Total pages	586	521	532

On January 1, 1944, there were 14 manuscripts on hand and during the year 44 additional papers were received. Of this total (58), 46 were published, 3 were returned to their authors, and 9 were left on hand at the end of the year. Of the 9 manuscripts left on hand, 7 are published in the February 1945 issue and 2 in the April issue. There is a marked decrease in manuscripts received but, since this is common to all scientific periodicals during the war period, there is no cause for alarm.

The printer is devoting about 40% of his production to war contracts. Since many of these contracts call for immediate processing, our journal has been given low priority rating on the schedule of Waverly. The editor hopes that this can be corrected in the near future.

Contributions continue to be markedly restricted to a small number of dental schools. The papers published in Volume 23 came from only 13 dental schools. The leading contributing institutions are given below (with 1941 for comparison).

Institution	*1944*	*1941*
University of Rochester*	5	9
Northwestern University	4	1
Columbia University	3.5	14
University of Chicago*	3	4
Baylor University	3.5	1

* No dental school.

The need for better distribution of research work is evident from a review of the contributing institutions. Although the Journal is not the exclusive publishing medium for the results of investigations in the dental field, the contributions to it may be used as an indication of trends.

May 24, 1945

HAMILTON B. G. ROBINSON, *Editor.*

Report of the Business Manager From January 1, to December 31, 1944

Statement for Fiscal Year 1944

Balance on December 31, 1943		$2,399.22
Receipts		
Subscriptions and Single Copies, Advertising, Extra Illustrations, Misc.	$7,708.82	
Total Receipts		$7,708.82
Disbursements		
Printing	$5,712.75	
Stationery and Supplies	66.90	
Postage	136.50	
Clerical and Misc.	1,313.46	
Total Disbursements		$7,229.61
Balance on hand December 31, 1944		$2,803.83

Respectively submitted,

J. FRANK HALL.

Report on the Circulation, 1944

Number of copies printed	2,450
Number of copies stored by printer	670
Number sent to members of I. A. D. R.	495
Number of regular paid subscriptions	875

Number of student subscriptions	16
Exchanges	99
Number sent to office of Business Manager for sale as single copies and sample copies	75
Number of foreign subscribers	80
Total number of Journals mailed out	1,780

Respectively submitted,
J. FRANK HALL.

C. WILLIAM J. GIES, ENDOWMENT FUND, JOURNAL OF DENTAL RESEARCH

Financial Statement as of May 22, 1945

This Fund was created, on the initiative of the voluntary Committee on Endowment—organized in New York City in 1937—to insure the continuance of the JOURNAL OF DENTAL RESEARCH "in full accord with the highest ideals of strictly professional journalism devoted to the advancement of research," to which the JOURNAL was dedicated at its establishment in 1919.

I. Amounts received from organizations (March 17, 1944 to May 22, 1945)

Alpha Omega Fraternity	$25.00	
American Association for the Advancement of University Education in Dentistry	70.00	
American College of Dentists (completes pledge of $5,000)	1,000.00	
Maryland Section	100.00	
Eastern Shore Dental Society (Maryland)	25.00	
International Association for Dental Research—American Division	192.45	
J. B. Mann Study Club	100.00	
Milwaukee County Dental Society (Wisconsin)	25.00	
Pawtucket Dental Society (Rhode Island)	25.00	
Peoria District Dental Society (Illinois)	25.00	
Rhode Island State Dental Society	50.00	
Southern Maryland Dental Society	50.00	
Wisconsin State Dental Society	165.00	
		$1,852.45

II. Amounts received from individuals (March 17, 1944 to May 22, 1945)

$150: E. George Meisel, Arthur H. Merritt (2 × $150)	$300.00	
$125: Archie A. Albert (1 × $125)	125.00	
$100: Norman H. Fortier (1 × $100)	100.00	
$50: Philip J. Conley, Bruce Thomas (2 × $50)	100.00	
$25: M. K. Baklor, Maurice A. Denby, Arthur M. Dring, Samuel Gorfine, Francis A. Holland, Leland D. Jones, Earl B. Keighley, Olin Kirkland, George J. Schreiber, Jr., Sidney Sorrin, Haides Weeks (11 × $25)	275.00	
Contributions from organizations carried forward		$1,852.45
$20: J. F. Cart, Clarke E. Chamberlain, George Wood Clapp, Thomas W. Clune, Charles D. Hermon, James Kershaw, W. S. Peters, C. M. Smith, R. C. Willett (9 × $20)	$180.00	
$15: E. M. Clifford, Hugh T. Hicks, Holly C. Jarvis, Arthur M. Johnston, Otto B. Litweiller, Elmer E. Megaw, George B. Paffenbarger (completes pledge of $250), J. A. Sinclair (8 × $15)	120.00	

$10: E. L. Adams, R. A. Adams, O. C. Barber, Albert A. Beausoliel, Ilie Berger, R. J. Broussard, Walter C. Chapin, E. A. Charbonnel, O. L. Colee, Thomas Conner, Harold J. Cronin, Maxwell M. Dixon, L. M. Edwards, A. R. Fogg, Lewis Fox, Edward L. Gambill, William Gee, C. L. Gowen, Henry Helfand, Ernest H. Henrichs, William A. Hillis, Edwin A. Holbrook, R. E. Huntington, George Karn, George P. Kier, William Kress, Arthur Lankford, Harry Levins, Morton J. Loeb, Virgil Loeb, J. L. Loftus, Ambrose Lynch, A. W. Maier, Joseph P. Massicotte, James W. McCarl, Frederick S. McKay, Charles F. McKivergan, William J. Meier, I. S. Miller, Edward C. Morin, William A. Morinville, C. W. Morrison, Donald F. Mosher, Emil Mueller, Stevens G. Nicholson, Walter L. Oggesen, Donald Osborn, Theodore O. Peterson, Kryle W. Preis, Dr. Ralston, Celia Rich, John Ronslin, William Rosenbaum, A. M. Schutzman, Byron N. H. Smith, Rowe Smith, Oliver F. Steber, David Tanchester, G. D. Timmons, John E. Tyler, C. W. Vivian, William R. Wright (62 × $10)	620.00	
$5: Charles C. Brown, Raymond W. Gatchell, Irving A. Hardy, William A. Jaquette, James C. Krasnoff, T. Way McDonald, A. L. McDonough, Alvin L. Miller, Evans H. Nelson, P. S. Neuwirth, Joseph Romenski, Ezra E. Schaefer, Ralph Stolworthy (13 × $5)	65.00	
$4.50: F. A. Richmond (interest on bond)	4.50	
$2.50: Thomas J. Clagett (1 × $2.50)	2.50	
Total contributions 1944–45		$3,744.45

III. Interest, Income and Expense (current year)

No expenses were recorded.

Number of bonds	*Denomination of bonds*	*Date Purchased*	*Interest accrued to date*	
10	$1,000.00	March 1942	$200.00	
22	1,000.00	May 1942	440.00	
8	1,000.00	July 1942	112.00	
2	1,000.00	Dec. 1942	18.00	
1	1,000.00	March 1943	9.00	
5	1,000.00	March 1944	10.00	
2	1,000.00	May 1944	4.00	
1	25.00	Feb. 1944	0.05	
			$793.05	$793.05
Total addition to the Fund as of May 22, 1945				$4,537.50

IV. Summary of total receipts (assets); 1937–45

Total receipts for the Fund, as of March 17, 1944		$36,369.22
Paid for 43 War Bonds (sec. VI)	$31,820.00	
Balance on deposit	4,102.22	
Bonds and shares	447.00	
Receipts in 1944–45		
From organizations (Sec. I)	1,852.45	
From individuals (Sec. II)	1,892.00	3,744.45
Interest accrued to date	793.05	793.05
		$40,906.72

V. Cumulative summary of annual recorded items of receipts and expenditures

	Contributions (payments)	*Interest*	*Expense*	*Net Receipts*	*Cumulative annual net receipts*
1937–38	$10,652.50	None	134.06	10,518.44	10,518.44
1938–39	4,748.17	None	None	4,748.17	15,266.61
1939–40	5,693.78	None	None	5,693.78	20,960.39
1940–41	5,190.55	206.78	3.53	5,393.80	26,354.19
1941–42	4,166.35	333.95	None	4,500.30	30,854.49
1942–43	1,766.31	112.19	None	1,878.50	32,732.99
1943–44	3,626.23	None	None	3,636.23	36,369.22
1944–45	3,744.45	793.05	None	4,537.50	40,906.72
Totals:	$39,598.34	$1,445.97	$137.59	$40,906.72	

VI. Statement regarding investment in War Bonds

By authorization of the Trustees of the Association, fifty War Bonds of Series F ("12-year appreciation bonds"), costing $740 each, have been purchased from the funds in the Endowment Committee's treasury, as of the following dates and serial numbers:

Purchased (10) March 1942: M140400F to M140409F, incl.	$7,400.00
Purchased (22) May 1942: M197811F to M197832F, incl.	16,280.00
Purchased (8) July 1942: M237558F to M237565F, incl.	5,920.00
Purchased (2) December 1942: M423990F and M423991F	1,480.00
Purchased (1) March 1943: M204356F	740.00
Purchased (5) March 1944: M750832F to M750836F, incl.	3,700.00
Purchased (2) May 1944: M752375F and M752376F	1,480.00
	$37,000.00

The payee named on each bond is "Committee on the William J. Gies Endowment Fund for the Journal of Dental Research (an unincorporated body)." All of these War Bonds (and the Bethany Methodist Hospital Bond and Farnsworth Television and Radio Corporation Stock, indicated in Section IV—report for 1943–44, are in the custody of the Treasurer of the Committee, in a safety deposit box, Empire Trust Company, 580 Fifth Avenue, New York City, in trust for the Committee.

Additional War Bonds will be purchased from the funds currently on deposit.

In 1943–44 there was a contribution from Dr. Joseph E. Psayala in the form of a $25 United States War Bond Series F ("12-year appreciation bond") Q631670F, the payee being "William John Gies Endowment Fund for the Journal of Dental Research." It is in the custody of the Treasurer in a safety deposit box, Empire Trust Company, 580 Fifth Avenue, New York City, in trust for the Committee, the bond was purchased in February 1944.

VII. Summary of open accounts relating to pledges

A. Organizations

	Pledge	*Paid*	*Balance*	
American College of Dentists	$5,000	$5,000	—	
International College of Dentists	800	200	600	
Kansas: First District Dental Society	100	25	75	
J. B. Mann Study Club, Washington, D. C.	1,000	200	800	$1,475.00

B. Individuals

Harry Kaplan	250	100	150	
George B. Paffenbarger	250	250	—	
R. J. Rinehart	200	75	125	275.00
				$1,750.00

L. W. Waugh, *Treasurer,*
William J. Gies Endowment Fund for the Journal of Dental Research.

D. OFFICERS AND MEMBERS ELECTED

ACTIVE OFFICERS: President—*Wallace D. Armstrong*, University of Minnesota. President-elect—*Samuel W. Chase*, Western Reserve University. Vice-president—*Harold C. Hodge*, University of Rochester. Secretary-treasurer—*Edward H. Hatton*, Northwestern University. Trustee (for a 5 year term)—*Edward H. Hatton*.

MEMBERS ELECTED: Bethesda, Md.—*James L. Bradley* and *E. M. Short*, National Institute of Health. Boston, Mass.—*Joseph P. Lazansky*, Tufts College. Chicago, Ill.—*Allan O. Gruebbel*, American Dental Association; *G. W. Rapp*, Loyola University; *Carlos E. Pomès*, Northwestern University; *William F. Barnfield*, University of Illinois; *Leroy E. Kurth*, U. S. N., Dental Corps, and *Avery S. Krashen*, U. S. A., Dental Corps. Iowa City—*Julian D. Boyd*, Iowa State University. Lincoln, Neb.—*Donald A. Keys*, University of Nebraska. Louisville—*Philip E. Blackerby*, Kellogg Foundation. Rochester, Minn.—*Stanley Lovestedt*, Mayo Foundation. Rochester, N. Y.—*Paul H. Keyes*, University of Rochester. San Antonio—*Beryl T. Ritchie*, U. S. A., Dental Corps. San Francisco—*Harry E. Frisbie*, University of California and *Henry M. Leicester*, College of Physicians and Surgeons.

E. MEMBERS IN ATTENDANCE

Ann Arbor.—Peyton. *Chicago*—Blackberg, Brodie, Burrill, Burwasser, Calandra, Cooper, Dahlberg, Denton, Fosdick, Harrison, Hatton, Hemmens, Kesel, F. B. Noyes, H. J. Noyes, Orban, Schram, Shell, Sicher, Skillen, Stephan, Teuscher, Tilden, Tylman, Wallace, Wassermann, Weinmann. *Cleveland*—Broadbent, Chase. *Columbus*—Robinson. *Indianapolis*—Hall. *Minnesota*—Armstrong. *Pittsburg*—Cox. *Rochester*—Hodge, McCauley. *San Francisco*—Hollenbeck. *Tennessee*—Jasper. *Washington*—Dean. Total, 39.

F. MEMBERS VOTING AT ANNUAL MEETING

Aisenberg, G. M. Anderson, Applebaum, Appleton, Archer, Armstrong, Arnim, Arnold, Atkinson, Austin, Bauer, Bear, Becks, Berger, Bernier, Beube, Bevelander, Bibby, Bier, Blackwell, Bodecker, Boucher, Boyle, Brandhorst, Breitner, Broadbent, Bunting, Burkett, Burrill, Carr, Chase, Cheyne, Clawson, Clough, Cohen, Cox, Crawford, Crowell, Dahlberg, Dale, Davis, H. T. Dean, Marguerite Dean, Deatherage, Dietz, Dobbs, Easlick, East, Easton, L. F. Edwards, Ellis, Elvove, Sidney Epstein, Erikson, Fairbank, Figg, Fleming, Fosdick, Fox, C. W. Freeman, Gabel, Gafafer, Garrison, Ginn, Goldman, Gottlieb, Grossman, Hadley, Hall, Hansen, Hatton, Hayes, H. J. Healey, Hill, Hodge, Holliday, Hooper, Hubbell, Hughes, Hutchinson, Jasper, L. R. Johnson, Jump, Kaplan, Kelsey, Kern, Kerr, Kimball, Kingery, Kitchin, Klaffenbach, Klein, Kniessner, Koch, Krasnow, Krogh, Lalonde, Land, Lasby, Leavitt, Lefkowitz, Lischer, Majerison, Manly, A. W. Mann, Margolis, Mason, Massler, McBride, McCall, McCauley, McClendon, McClure, Merkeley, Merritt, Midgeley, Milhon, Millberry, S. C. Miller, Miner, G. R. Moore, Mary Moore, A. H. Mueller, Emil Mueller, R. E. Myers, V. C. Myers, C. A. Nelson, Neuwirth, Newman, Norwold, F. B. Noyes, H. J. Noyes, Nuckolls, Oblatt, O. A. Oliver, Oppenheim, Orban, O'Rourke, Paffenbarger, C. E. Palmer, Peyton, Phillips, Price, Prinz, Rabkin, Rault, Restarski, H. B. G. Robinson, A. P. Rogers, Rosebury, Romnes, Rosenstein, Scherer, Schour, E. W. Schultz, Schapiro, Shell, Sicher, Silberstein, Simmonds, Sinclair, Skillen, Skinner, Sloman, R. V. Smith, Speidel, Stafne, Steadman, Stephan, Stone, Henry Swanson, Sweet, W. E. Taylor, Teuscher, Thoma, B. O. A. Thomas, H. S. Thompson, G. E. Thompson, J. R. Thompson, Treloar, Volker, Volland, Wainwright, Waite, Waldron, Ward, Waugh, Weatherford, Wheeler, Willett, Willman, Winter, F. J. Wolfe, W. L. Wylie, Jr., Zegarelli, Ziskin. Total, 198.